Original Article

Comparison of Serum Cyclooxygenase-2 Level between Melasma and Nonmelasma Patients in Dr. Saiful Anwar General Hospital, Malang, Indonesia

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Abstract

Background: Melasma is the hypermelanosis skin disease on the sun exposed area. Ultraviolet exposure leads to inflammation in the epidermis and dermis, one of which is marked by an increase in Cyclooxygenase-2 (COX-2). COX-2 expression involves in the production of prostaglandin-E2 (PGE2) that take part in tyrosinase activation and melanogenesis. **Aims and Objectives:** This study aimed to determine the differences in serum COX-2 levels in melasma and non-melasma patients in Dr. Saiful Anwar General Hospital Malang, Indonesia. **Materials and Methods:** A cross-sectional study using continuous sampling in melasma and non-melasma patients at the Dermatology and Venereology out-patient clinic from November to December 2017. The COX-2 serum levels examined by ELISA method. **Results:** From the 23 melasma and 23 non-melasma subjects, the mean value of serum COX-2 levels in the melasma and nonmelasma groups was not significantly different ($P > 0.05$) with value of 82.23±61.08U/L and 52.66±28.62 U/L, respectively. Those might be influenced by the other unknown variables who were not included in this study. Based on Melasma Severity Score (MSS), serum COX-2 levels differed significantly in moderate severity (49.55±14.26 U/L) and severe (112.1±72.32 U/L) ($P > 0.05$) might related to the capacity of the enzyme that induces epidermal hyperpigmentation. **Conclusion:** There were differences in COX-2 levels in melasma and non-melasma patients, but the difference was not statistically significant. However, there is a tendency that as the COX-2 level increases, so as the severity of melasma. Therefore, the severity of melasma possibly influenced by inflammation markers.

Keywords: Cyclooxygenase-2 serum, exposure, melasma, sun, ultraviolet

Introduction

Melasma is a hypermelanosis skin disease of the sun-exposed area, especially face. Melasma indicated by macules and patches of irregular shapes in light brown to dark brown.[1,2]

Melasma often occurs in Asian, Oriental, and Hispanic races as well as in Fitzpatrick Type III–VI skin.[3,4] In Indonesia, the female-to-male ratio of the disease is 24:1, especially in women of childbearing age with a history of direct exposure to ultraviolet (UV) light in prolonged intensity.[5]

The pathogenesis and causes of melasma remain unclear, and the therapy is still a challenge.[2] The development of melasma is influenced by many factors and depends on environmental interactions (UV exposure), hormones, and genetic predispositions.[4] Melasma is triggered by subclinical inflammation which is induced by UV radiation and regulated by genetic and hormonal factors.[2,6,7] UV exposure to skin can increase the expression of cyclooxygenase-2 (COX-2) and increase the production of prostaglandin E2 (PGE2).[8] PGE2 has an essential role in the activation of tyrosinase and melanogenesis.[9] This is according to Rodríguez-Arámbula *et al*. with histopathological results that significantly increased COX-2 and interleukin-17 (IL-17) in melasma lesions compared to normal skin, where COX-2 can play a role directly or indirectly

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in the pathogenesis of melasma through tyrosinase activation and melanogenesis. COX-2 and IL-17 act synergistically to prolong the inflammatory conditions in melasma.[10] Identification of factors associated with the pathogenesis of melasma is expected to show the new targets for more efficient melasma therapies and better prevention of recurrence.[11] Thus, the authors need to determine the role of inflammation in the pathogenesis of melasma by measuring COX-2 levels.

However, there is no previous study about the comparative assessment of serum COX-2 levels in patients with melasma and nonmelasma. While measurement of COX-2 serum levels is more acceptable to patients than measurements through the biopsy of melasma tissue. Thus, this study aimed to examine COX-2 levels in melasma and nonmelasma patients and determine their role in the pathogenesis of melasma and as an alternative therapeutic target in the future.

Methods

Research design

This was a cross-sectional analytic observational study conducted at Dr. Saiful Anwar General Hospital, Malang, Indonesia, on November–December 2017. The sample collection is conducted according to consecutive sampling method and was approved by the Ethics Committee of Dr. Saiful Anwar General Hospital (No. 400/168/K.3/302/2017).

Participants' enrollment

The participants of this study included women patients in the age group of 21-55 years old who attending the Cosmetodermatology Division of Outpatient Department of Dermatology and Venereology. All patients were informed about the study procedures, risks, and benefits. The participants who opted to take part were included in the study after signing an informed consent form. The patients who met the inclusion criteria were the research participants. Patients were excluded from this study if they met the exclusion criteria. All participants were examined for their demography characteristics including age, gender, skin type, pattern of melasma, sun exposure duration, sunblock usage, and duration.

The inclusion criteria for melasma patients were women aged 21–55 years with a diagnosis of melasma based on medical history and clinical features and score of the Modified Melasma Area and Severity Index (mMASI) above 5.8. The inclusion criteria for nonmelasma participants were women aged 21–55 years, without skin disorders (i.e., melasma or other mild skin disorders).

The exclusion criteria were other patient conditions based on history taking and medical records. Those conditions include (1) systemic diseases that can affect COX-2 levels (malignancy, autoimmune diseases, diabetes mellitus, and kidney failure); (2) suffering from other skin diseases that affect serum COX-2 levels (skin malignancy, vitiligo, lichen planus, and psoriasis); (3) currently using topical corticosteroid melasma therapy and topical bleaching agent for the past 1 week (pregnant, breastfeeding, or menopause); (4) currently using hormonal contraception or estrogen/progesterone hormone replacement therapy for the past 2 months; (5) taking drugs (antimalarials, tetracycline, minocycline, doxycycline, anticonvulsants, amiodarone, antipsychotics, angiotensin-converting enzyme inhibitors, diuretics, and sulfonylureas which can affect the appearance of melasma) for the past 1 week; and (6) currently receiving oral treatment of corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs) (salicylates, propionic acid derivatives, acetic acid derivatives, enolic acid derivatives, fenamic acid derivatives, and COX-2 selective inhibitor) in the past 1 month.

Scoring of the Modified Melasma Area and Severity Index

All melasma patients were measured for determining mMASI score which was expressed in Equation 1 according to Pandya *et al*. Scores of mMASI were grouped according to the Melasma Severity Score (MSS), which include mild (2.7–4.9), moderate (5.8–7.2), and severe (8.0–9.8).[12]

$$mMASI = \left(0.3 \times A(f) \times \left(D(f) + H(f)\right)\right) + \left(0.3 \times A(lm) \times \left(D(lm) + H(lm)\right)\right) + \left(0.3 \times A(c) \times \left(D(c) + H(c)\right)\right)$$

Where A: Area of involvement, D: Darkness, H: Homogeneity of: (f): Forehead, (lm): Left malar, (c): Chin.

However, mMASI score would be used in this study above 5.8. Thus, the patients with moderate and severe levels were chosen as the research participants, but the mild had to be eliminated/excluded.

Collection of blood samples

Five milliliters of venous blood was taken from the cubital vein of melasma and nonmelasma (control) patients and allowed it to clot in the plain tube at room temperature. The serum was aspirated after centrifugation at 3000 rpm for 20 min. It was divided into few aliquots in plastic tubes and then stored (−80°C) until the time of estimation.

Determination of cyclooxygenase-2 serum level using enzyme-linked immunosorbent assay

Examination of blood serum COX-2 levels was made using the RayBio® Human COX-2 (Cyclooxygenase 2) ELISA kit. This assay employs an antibody specific for human COX-2 coated on a well plate. Standards and samples were pipetted into the wells, and COX-2 presented in a sample was bound to the wells by the immobilized antibody. The wells were washed, and biotinylated anti-human COX-2 antibody was added. After washing the unbound biotinylated antibody, horseradish peroxidase-conjugated streptavidin was pipetted to the wells. The wells were washed again, and 3,3',5,5'-tetramethylbenzidine chromogenic substrate solution was added to the wells and the color developed which indicated the amount of COX-2 bound. The solution changed the color from blue to yellow, and the intensity of the color was measured at 450 nm using ELISA reader board 550.

Statistical analysis

Data collection sheet was processed using the Statistical Package for Social Sciences (SPSS) version 20 (IBM Corp, United States). Comparison test was carried out using the unpaired *t*-test formula if the distribution was normal, whereas nonparametric test using the Mann–Whitney test if the data distribution was not normal ($P < 0.05$).

Results

Demographic conditions of research participants

From a total of 1593 (11.61%) melasma patients attending the Cosmetodermatology Division of Outpatient Department of Dermatology and Venereology, Dr. Saiful Anwar General Hospital, Malang, Indonesia, in 2016, 185 patients were enrolled as the study participants. The research has been conducted on 23 patients of melasma and 23 nonmelasma participants. The mean age of melasma patients was 47.60 years (standard deviation [SD] ± 6.27) and nonmelasma participants was 42.35 (SD ± 7.64) ($P = 0.019$). Fitzpatrick's skin types, demographic data, family history, sun exposure duration, sun exposure duration time, sunblock usage, sunblock usage duration, and sunblock usage duration time are shown in Table 1. Melasma patients had dominantly positive melasma family history. Sun exposure duration of melasma patients was below 6 h at the time between 09.00 a.m. and 03.00 p.m. By the same duration, the nonmelasma patients were exposed at the time below 09.00 a.m. Most of the melasma patients had used sunblock after melasma happened with usage duration below 6 h/day at 09.00 a.m. until 03.00 p.m. However, nonmelasma had a good prevention using sunblock before melasma symptoms.

Serum cyclooxygenase-2 levels of melasma and nonmelasma

The levels of COX-2 between melasma and nonmelasma patients are shown in Table 2. The ranges of serum COX-2 levels of the melasma and nonmelasma groups were 35.67–238.89 U/L and 23.56–150.11 U/L, respectively. The mean of COX-2 level of melasma and nonmelasma patients was not significantly different ($P > 0.05$). Figure 1 shows a melasma patient with centrofacial- and epidermal-type melasma and had mMASI score about 9.1 (severe melasma).

Severity of melasma based on the Modified Melasma Area and Severity Index score

The total mean value mMASI score of serum COX-2 levels is presented in Table 3, whereas Table 4 presents the comparison of serum COX-2 levels based on the MSS. Based on the mMASI score grouped according to MSS, there were 11 people in the moderate group (mMASI score: 5.8–7.2) and 14 people in the severe group (mMASI score: 8.0–9.8).

Type of melasma and effect of sunblock and ultraviolet exposure against serum cyclooxygenase-2 levels

Based on the type of melasma, the groups of melasma patients were categorized as follows: 5 – epidermal type, 1 – dermal type, and 17 – mixed type. The mean serum COX-2 levels in the melasma group based on melasma type are presented in Table 5. Serum COX-2 levels did not show significantly

Table 1: Demographic condition of research participants ($n = 23$)

Condition	Category	Melasma, *n* (%)	Nonmelasma, *n* (%)	*P*
Age	-	47.60±6.27	42.35±7.64	0.019*
Fitzpatrick's skin type	III	2 (8.6)	2 (8.6)	-
	IV	19 (82.6)	17 (73.9)	
	V	2 (8.6)	4 (17.3)	
Melasma family history	Negative	8 (34.8)	11 (47.8)	0.369
	Positive	15 (65.2)	12 (52.2)	
Sun exposure duration	<6 h	19 (82.6)	20 (86.9)	-
	>6 h	4 (17.4)	3 (13.0)	
Sun exposure duration time	<09.00 A.M	2 (8.7)	8 (34.8)	-
	09.00 A.M-03.00 P.M	16 (69.6)	6 (26.1)	
	<09.00 A.M-15.00 P.M	4 (17.4)	1 (4.4)	
	<09.00 A.M, >15.00 P.M	2 (8.7)	9 (39.1)	
Sunblock usage	Never	10 (43.5)	13 (56.5)	-
	Before melasma	3 (13.0)	10 (43.5)	
	After melasma	10 (43.5)	0 (0.0)	
Sunblock usage duration	Never	10 (43.5)	12 (52.1)	-
	<6 h	8 (34.8)	10 (43.5)	
	>6 h	5 (21.7)	1 (4.4)	
Sunblock usage duration time	Never	10 (43.5)	13 (56.5)	-
	<09.00 A.M	8 (34.8)	10 (43.5)	
	09.00 A.M-03.00 P.M	5 (21.7)	0 (0.0)	
	<09.00 A.M, >03.00 P.M	0 (0.0)	0 (0.0)	

*There were different significantly among melasma and nonmelasma category condition

different ($P > 0.05$) according to melasma type (epidermal, dermal, and mixed).

The differences in serum COX-2 levels in melasma and nonmelasma patients in some UV exposure and the use of sunscreen are shown in Table 6. Most of the conditions which may cause dynamics of COX-2 levels did not show the significant difference although most of the conditions showed slightly higher in melasma patients than nonmelasma participants. However, the usage of sunblock in melasma patients will significantly produce COX-2 level higher than nonmelasma patients.

Table 2: Value of serum cyclooxygenase-2 levels of melasma and nonmelasma patients

Patients	Serum COX-2 level (U/L)			
	Range	Median	Mean±SD	*P* value of mean
Melasma	35.67-238.89	52.66	82.23±61.08	0.063*
Nonmelasma	23.56-150.11	44.00	52.66±28.62	

*Serum COX-2 levels between melasma and nonmelasma patients showed different significantly. COX: Cyclooxygenase-2, SD: Standard deviation

Table 3: Comparison of Modified Melasma Area and Severity Index value and serum cyclooxygenase-2 levels in melasma patients

	Lowest	Highest	Mean±SD
mMASI score	6.80	15.30	9.63±2.73
COX-2 melasma level	35.67	238.89	82.23±61.08

mMASI: Modified Melasma Area and Severity Index, COX-2: Cyclooxygenase-2, SD: Standard deviation

Table 4: Comparison of serum cyclooxygenase-2 levels according to Melasma Severity Score

MSS category	Serum COX-2 level		
	Mean±SD	Median	*P* value of mean
Moderate	49.55±14.26	44.89	0.051
Severe	112.19±72.32	104.72	

SD: Standard deviation, MSS: Melasma Severity Score, COX-2: Cyclooxygenase-2

Discussion

The exact of melasma cause remained unknown despite many factors involved in this disease pathogenesis.[13] The study about melasma is complex yet focused on the examination of the basic biochemistry, pharmacology, and physiology of the melanocortin system, the development of melanosomes, genetics, diseases associated with abnormal pigment, and environmental exposure to chemical materials.[14] To improve the understanding of the pathogenesis, scientists have to master the genomic first and basic proteomic melasma, including hundreds of proteins involved in pigmentation.[15]

A study by Passeron and Picardo (2018) suggested the latest evidence on the pathophysiology of melasma and suggested that melasma might be a photoaging skin disorder affecting genetically predisposed individuals.[16] Various factors including UV light exposure and melasma history family have a possible impact on the development of melasma in almost all patients.[17] UVA, UVB, and sunlight can affect the process of melanogenesis, but the involvement of hormones is also essential in the difficulty of melasma.[3,6,18-20] Solar irradiation with UVB (280–315 nm) and UVA (315–400) and the shorter wavelengths of visible light stimulate these cells to promote melanogenesis and melanocyte proliferation. The main effects of acute and chronic exposure to UV radiation are DNA damage, inflammation, and immunosuppression.[21] The whole array of changes caused by UV radiation in exposed skin is termed as photoaging. A primary cause of aging is the imbalance between reactive oxygen species production and their neutralization by natural antioxidant systems, which generates oxidative stress leading to the progressive deterioration of the organs and its resultant clinical and histological changes.[21-23] Acute exposure is known to trigger worsening or relapses of melasma lesions. Chronic exposure, especially of UVA1 and visible light that penetrate deeper into the skin, might chronically affect the basal membrane and the dermis component to induce, in genetically predisposed patients, the melasma lesions.[16]

In this study, the mean age for the melasma and nonmelasma groups was 47.60 ± 6.27 and 42.35 ± 7.64 years, respectively.

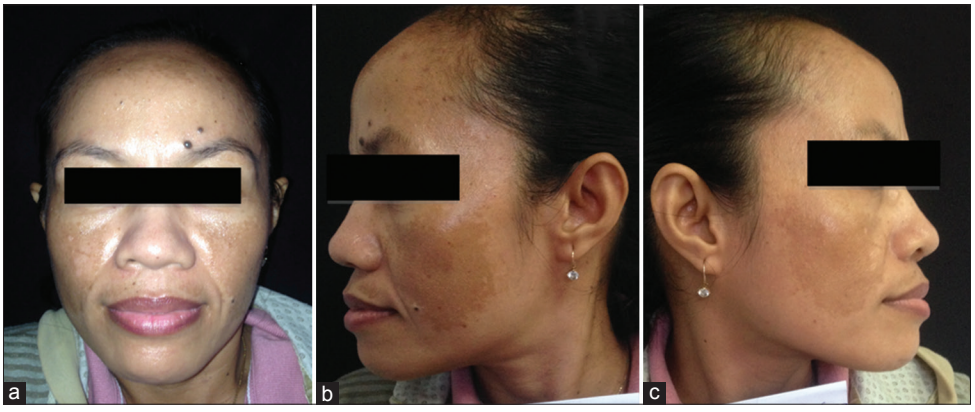

Figure 1: One of the melasma patients' appearance in this study from (a) front, (b) left, and (c) right

Similar with Ortonne *et al*., in nine clinics spread across the world obtained a mean age of 42.9 ± 9; in the United States 45.0 ± 10.7, in France 41.0 ± 7.46, in Germany 35.1 ± 7.18, in the Netherlands 40.7 ± 8.86, in Mexico 39.5 ± 7.77, in Italy 41.3 ± 5.91, in Singapore 48.7 ± 6.71, in South Korea 37.5 9.33 and in Hong Kong 48.7 7.83.[17]

The beginning of melasma lesions is indicated by the impaired integrity of the stratum corneum, slower repairability, and an increase of inflammatory cells in the development of melasma lesions in Asian skin.[24,25] Histologically, a positive correlation was found between COX-2 immunohistochemical staining with solar elastosis and melanin in the epidermis.[10] COX-2 expression induced by UV exposure involves inflammation due to UV exposure, edema, keratinocyte proliferation, and epidermal hyperplasia.[26] COX-2 will affect the local inflammatory response through action on immune cells.[27]

Melasma patients in this study were women aged 21–55 years with a diagnosis of melasma based on medical history and clinical features and mMASI score >5.8. A study by Pandya *et al.* sought to stratify the mMASI into ranges correlating with mild, moderate, and severe melasma, so that clinicians can better interpret melasma studies and investigators can identify patients with moderate-to-severe melasma by correlating MSS categories to mMASI scores.[12] In this study, the mMASI score was used above 5.8 because we need to evaluate patients with moderate-to-severe melasma.[12]

The serum COX-2 levels in the melasma and nonmelasma groups were 35.67–238.89 and 23.56–150.11 U/L, respectively. The mean value of serum COX-2 levels of the melasma and nonmelasma groups was 82.23 ± 61.08 and 52.66 ± 28.62 U/L, respectively ($P = 0.063$). This is not by the study by Rodríguez-Arámbula *et al*. that COX-2 expression using immunohistochemistry on histopathology of melasma and nonmelasma lesions was significantly different ($P < 0.001$).

Table 5: Differences in serum COX-2 levels in melasma type

Melasma type	Mean±SD	Median	*P*
Epidermal	77.67±50.37	58.67	0.746
Dermal	140.45±135.14	72.72	
Mixed	76.38±55.53	52.67	

*P<0.05: there are significant differences based on Kruskal Wallis

In melasma and nonmelasma lesions, the mean value of COX-2 expression was 8.3 ± 2 and 6.2 ± 0.6, respectively.[10] This was probably due to an examination of COX-2 levels taken from serum that might have been influenced by other variables outside of the research. The COX-2 enzyme is in the smooth endoplasmic reticulum and bound to the cell's core membrane.[27] The measurement of COX-2 levels in serum and tissue certainly has a different result, whereas the measurement of COX-2 serum levels is more acceptable to patients than the measurements through the biopsy of melasma tissue. The biopsy of melasma lesion can be a cosmetical problem. Late "scarring" with or without hypo- or hyperpigmentation is a common complication seen after healing of the skin biopsy site. Hypopigmented scars are common when biopsies are taken for hyperpigmented lesions. Scars can be atrophic scar or hypertrophic. Occasionally, patients may develop a keloid over the biopsy site.[28]

The previous reported that the most common cause of melasma is a combination of UV exposure, genetic tendency, and hormonal influences. Moreover, there are still many systemic factors that can affect both melasma and serum COX-2 levels that have not been included in the exclusion criteria in this study. Systemic disease factors that can affect melasma are endocrine disorders, liver disease, and nutritional deficiency.[1]

COX-2 is regulated by growth factors, light, and cytokines and is likely to be involved in the inflammatory process due to UV, photoaging, and photocarcinogenesis.[29] Repeated UV exposure is known to cause a chronic increase in expression of PGE2 which is induced by COX-2.[9] IL-17 induces COX-2 synergistically to prolong the inflammatory state in melasma. High levels of IL-17 in the epidermis in melasma lesions can be the main key to the persistence of melasma.[10]

The COX-2 enzyme is usually not present in basal conditions or may be in deficient amounts. The COX-2 enzyme is rapidly induced by various stimuli, including proinflammatory cytokines, such as IL-1, tumor necrosis factor-alpha, and growth factors, to produce prostaglandin synthesis associated with inflammation and carcinogenesis. Substantial evidence suggests that irregular COX-2 expression and prostaglandin synthesis affect chronic inflammatory conditions.[30] Systemic diseases that can affect serum COX-2 levels are a malignancy, systemic lupus erythematosus, systemic sclerosis, rheumatoid arthritis, atherosclerosis, diabetes mellitus, multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's

Table 6: Differences in serum COX-2 levels of melasma and non-melasma patients in some conditions

Condition	COX-2 Level (U/L)		Significancy test (*P*)
	Melasma	Non Melasma	
UV Exposure <6 h (*n*=39)	83.88±63.17 (*n*=19)	54.07±30.01 (*n*=20)	0.101
UV Exposure >6 h (*n*=7)	74.39±57.63 (*n*=4)	43.26±17.31 (*n*=3)	0.400
Exposure time 09.00-15.00 (*n*=22)	63.49±51.89 (*n*=16)	39.53±12.79 (*n*=6)	0.275
Did not using sunblock (*n*=23)	119.23±73.04 (*n*=10)	51.52±31.20 (*n*=13)	0.005*
Use sunblock before melasma (*n*=14)	42.33±6.01 (*n*=3)	61.40±34.77 (*n*=11)	0.573

*P<0.05: there are significant differences based on the Mann Whitney test

disease, inflammatory bowel disease, chronic hepatitis, liver cirrhosis, osteoarthritis, and failure of galaxies.[31-33] Some of these systemic diseases have not been included in the exclusion criteria in this study.

The average total score of mMASI in this study was 9.63 ± 2.73, with the lowest and highest scores being 6.8 and 15.3, respectively. Based on MSS, serum COX-2 levels were found at a moderate level of 49.55 ± 14.26 U/L and a severe level of 112.19 ± 72.32 U/L ($P = 0.05$). According to Rodríguez-Arámbula *et al*. investigation, COX-2 was thought to have a direct relationship with the pathogenesis of melasma based on the result that the mMasi score was positively related to T-cells and COX-2 expression. The expression of COX-2 and the severity of melasma may be related to the capacity of the enzyme that induces epidermal hyperpigmentation through prostaglandin production in the photoaging state played primarily by chronic inflammatory cells and mediators.[10]

Some limitations in this study that possibly cause bias include COX-2 examination from blood serum, where COX-2 levels in blood serum are influenced by several factors. The results of COX-2 levels in this study do not necessarily describe COX-2 levels derived from melasma but also can be possible from other factors in the body. In this study, exclusion was carried out with various conditions that could lead to an increase in serum COX-2 but only based on medical history. The method to measure melasma severity uses the mMASI method that is measured subjectively so that it needs more objective examination such as Mexameter or Chromameter. It is necessary to research with histopathological examination on biopsy results of melasma skin lesions and normal skin. In order to obtain more accurate results, it is necessary not only to review the medical history but also physical and laboratory examinations to rule out systemic diseases that can affect serum COX-2 levels.

According to a research by Rodríguez-Arámbula *et al*., the presence of COX-2 involvement in melasma may explain the good response to the treatment of topical anti-inflammatory drugs.[10] In the study of Jung *et al*., the clinical efficacy of madecassoside (the main triterpene glycoside isolated from *Centella asiatica*) significantly reduced melanin index due to UV exposure in the 8th week after topical application.[34] However, there has been no research on the use of systemic COX-2 inhibitors in melasma. Another study by Kim *et al*. explains that COX-2 is suspected to be the target candidate for the development of therapeutic antimelanogenic agents or lightening agents for hyperpigmentation disorders such as melasma, postinflammatory hyperpigmentation, and solar lentigo.[9]

There has been no study of COX-2 systemic drug application in melasma, but there are studies in other diseases, namely aspirin (acetylsalicylic acid) and other NSAIDs (indomethacin, piroxicam, sulindac, diclofenac, and celecoxib) which are useful for reducing skin cancer incidence and as therapy actinic keratosis.[35,36] The molecule decreases prostaglandin production by inhibiting COX-1 and COX-2, whereas celecoxib is a specific COX-2 inhibitor.[37,38] Therefore, further research is needed on COX-2 inhibitors as candidates for melasma therapy agents.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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