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Relationship between Thioredoxin Reductase (TrxR) Levels and Melasma

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ABSTRACT

Introduction: Melasma is a common condition of acquired hypermelanosis in dermatology that often occurs in areas of the body exposed to sunlight, especially the face. Thioredoxin Reductase (TrxR) is a key antioxidant system in defense against oxidative stress through disulfide reductase activity that regulates dithiol/disulfide protein balance. High levels/activity of TrxR correlate with melanin formation and tyrosinase activity which provides additional information about the role of cellular antioxidant proteins in melanogenesis which is suspected to be related to the occurrence of melasma. The aim of this study was to determine the relationship between TrxR levels and melasma

Method: This study was an observational analytical study with cross-sectional design on 30 melasma patients and 30 controls. Each patient underwent anamnesis, dermatological examination, and blood sampling to assess TrxR levels by ELISA test. These data were analyzed statistically using the Mann Whitney test.

Results: This study shows that the most common distribution pattern of melasma was centrofacial in 24 people (80%). The mean TrxR level in melasma was 12.73±11.66 ng/ml. The results of the study showed that there was a relationship between high TrxR levels and melasma ($p<0.001$). The TrxR mean level based on the duration of suffering from melasma at <5 years was 11.4±2.89, and ≥ 5 years was 14.0±3,18

Conclusion: There is a relationship between TrxR levels and melasma.

Keywords

Melasma, Thioredoxin reductase, TrxR levels, Chloasma, Antioxidant

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INTRODUCTION

Melasma is a common condition of acquired hypermelanosis in dermatology that often occurs on areas of the body exposed to sunlight, especially the face. Melasma predominantly affects women with Fitzpatrick skin types III–V, appears bilaterally, spreads symmetrically, has an irregular shape, characterized by dark brown macules and usually appears on the cheeks, forehead, nose or upper lip of the face with centrofacial, malar or mandibula distribution pattern,[1-3] In Indonesia, the prevalence of melasma is estimated at around 0.25–4% of all cases of skin disease.[4] Research by Jusuf at the Haji Adam Malik Hospital in Medan in 2012–2015 showed an increase in the prevalence of melasma from 78.85% in 2012, 83.78% in 2013, 66.67% in 2014 and 87.5% in 2015.[5]

Melasma is also considered a photoaging disorder, with findings of increased vascularity and mast cells, solar elastosis, and changes in basement membrane histology.[6] It has been widely discussed that oxidative stress, produced by ultraviolet radiation (UVR), causes long-term photoaging.[7] According to advances in medicine, the role of oxidative stress in the pathogenesis of various dermatological and nondermatological

disorders has also been hypothesized. The role of oxidative stress in melasma has also been studied further.[8,9] It is known that Reactive Oxygen Species (ROS) produced in the skin by UVR are a major contributor to oxidative damage to the skin. Excessive ROS production causes oxidative stress, resulting in cellular and physiological function abnormalities, causing damage to DNA, proteins, and lipids. Reactive Oxygen Species also regulate melanin synthesis through a number of mechanisms.[9,10]

The thioredoxin (Trx) system, consisting of Nicotinamide Adenine Dinucleotide Phosphate (NADPH), thioredoxin reductase (TrxR), and thioredoxin, is a key antioxidant system in defense against oxidative stress through disulfide reductase activity that regulates dithiol/disulfide protein balance.[11] There are also report that TrxR is highly expressed on the surface of human keratinocytes and melanocytes, which act as the skin's first line of defense against free radicals generated in response to UV light.[12,13]

It has been shown that reduced Trx suppresses melanin synthesis by reacting with the binuclear copper center of tyrosinase, thereby inhibiting tyrosinase activity. TrxR, a component of the Trx system, has also been reported to be involved in melanogenesis. High levels/activity of TrxR correlate with melanin formation, providing additional information about the role of cellular antioxidant proteins in melanogenesis. The function and expression of antioxidant enzyme activity such as glutathione reductase, glutathione peroxidase, thioredoxin reductase, and catalase most likely modify the melanosome pathway. In melasma conditions, it is thought that the TrxR levels found may also increase. The role and relationship of the thioredoxin system, especially thioredoxin reductase, in melasma is still unclear, however, the thioredoxin system plays a role in influencing tyrosinase activity and melanin formation which is related to the occurrence of melasma.[10]

Based on the explanation above, there is a hypothesis that TrxR has a role in the pathophysiology of melasma, but it is still limited, so research is needed to determine the relationship between TrxR levels and melasma.

METHOD

This study is an analytic observational study with a cross sectional design involving 18□50 years old of 30 melasma patients and 30 controls at the Dermatology and Venereology polyclinic in Universitas Sumatera Utara, Medan. Every research subject who informed consent was included in this study. The exclusion criteria were pregnant and lactating women, patients who have consumed antioxidant supplements in the last 1 month, cancer, metabolic syndrome, psoriasis, and neurodegenerative disease. The ethical license was granted by the Health Research Ethics Committee, Faculty of Medicine, Universitas Sumatera Utara.

This study was conducted after obtaining ethical clearance from the Research Ethics Committee of the University of Sumatera Utara (No. 271/KEPK/USU/2023) and from the Prof. Chairuddin P Lubis Universitas Sumatera Utara Hospital Research Permit (No. 1739/UN5.4. 1.1.3/KPM/2023).

The data collected by researchers of the patients who come to Dermatology and Venereology polyclinic in Universitas Sumatera Utara, Medan. Researchers is taking a careful history, clinical examination, and blood sample tests to check TrxR plasma. TrxR levels were measured by using Human TrxR Elisa Kit. The data collected were analyzed using Mann Whitney test to determine the relationship between TrxR plasma levels with melasma which $p < 0.05$ was considered a significant result.

RESULT

In this study, the subjects were divided into melasma and control groups (with no melasma). The characteristics of the subjects based on age were presented in Table 1. Most of the research subjects in the melasma and control group were 36–45 years old in 73.3% and 57.5% respectively. Table 2 shows the characteristics of subjects based on distribution pattern of melasma in this study. It was stated that the majority distribution pattern of melasma were centrofacial in 24 people (80%), followed by malar in 6 people (20%).

Then the TrxR level was measured using ELISA. The ELISA showed that TrxR level in the melasma group was higher than in the control group (12.73±11.66 ng/ml vs. 4.80±3.21 ng/ml). Assessing the relationship between TrxR level and melasma, we found that there was a relationship between high TrxR levels and melasma ($p<0.001$). (Table 3). Based

on the duration of suffering from melasma, there were 15 subjects (50%) who had suffered from melasma for <5 years and 15 subjects (50%) who have had melasma for ⩾5 years. According to the TrxR mean level based on the duration of suffering from melasma, mean TrxR levels in the melasma subject group suffering from <5 years was 11.4 ng/mL, while the mean Trxr level in the melasma subject group suffering from ⩾5 years was 14.0 ng/mL. (Table 4).

Table 1. Distribution of research subjects by age

Age (years)	Melasma patients		Control	
	n	%	n	%
18–25	0	0	0	0
26–35	3	10	12	40
36–45	22	73.3	13	43.3
46- 55	5	16.7	5	16.7
Total	30	100	30	100

Table 2. Distribution patterns in melasma subjects

Patterns	n	%
Centrofasial	24	80
Malar	6	20
Mandibular	0	0
Total	30	100

Table 3. Relationship between TrxR levels and melasma

Group	TrxR levels			*p value**
	n	Mean (ng/mL)	SD	
Melasma	30	12.73	11.66	<0.001*
Control	30	4.80	3.21	

**Mann Whitney*

Table 4. TrxR mean level based on the duration of suffering from melasma

Duration			TrxR Levels	
	n	%	Mean	SD
<5 years	15	50	11.4	2.89
≥5 years	15	50	14.0	3.18
Total	30	100		

DISCUSSION

Melasma has a variable onset of age. The average age range for melasma onset varies from 20–30 years in some studies to 36–40 years in several other studies.[6,7] This study found that most of the research subjects in the the melasma and control group were 36–45 years old and the youngest age was the 26– 35 years old group. Research conducted by Zulfa, Putra and Jusuf in Dermatology and Venereology Polyclinic, Universitas Sumatera Utara Hospital showed that the highest distribution of melasma was found in women aged 36-45 years, and another research by Jusuf reported that the highest age groups were 31-40 years and 41-50 years with an average age of 39.[3] years which is age group in which melasma is most commonly found.[5,14,15] Melasma has been proven develops early in life in patients with a lower phototype. The delay in the appearance of melasma has been attributed to its photoprotective role of melanin.[16]

According to the distribution pattern of melasma, this study found that the majority of melasma subjects were centrofacial in 24 people (80%), followed by malar in 6 people (20%), and no mandibular pattern was found in melasma subject in this study. The same condition were found in a study by Mahdalena, Jusuf, and Putra and in a study by Oluwatobi, Godec, and Elbuluk where the main clinical pattern in 50–80% of cases is centrofacial pattern, affecting the forehead, nose, and upper lip followed by malar pattern which limited to the cheeks.[17,18] Studies in India and Brazil also showed that centrofacial was the most common type of melasma (69.2%) followed by malar (43.4%). Similar findings were found in Tunisia, where centrofacial melasma is the most common common (76.1%) among 197 patients, and malar and mandibular type melasma found in 22.9% and 1%, respectively.[19] This matter possibly due to the larger centrofacial area (forehead, chin, cheeks, nose, upper lip, etc) exposed to sunlight compared to other distributions. However different to those

found in Brazil, research in Singapore shows melasma with a mandibular distribution pattern is the most common type. Difference some of these studies primarily focus on ethnic and geographic differences.[20]

In this study there was an increase in the mean value of TrxR levels in the melasma group which is 12.73 ng/mL, while in the control group there was a decrease in TrxR levels with a mean of 4.80 ng/mL. Based on the results of the analysis, it is concluded that there is a significant relationship between TrxR levels and melasma. TrxR values in melasma groups tended to be higher than the control group. In accordance with the literature, the same condition was found in the research by Lu et.al that showed high TrxR levels/activity correlate with melanin formation, which provides additional information about the role of protein cellular antioxidants in melanogenesis.[10] Cell responses to oxidative stress conditions include an increase in antioxidant capacity and upregulation in signaling pathways contributes to defense against oxidative stress and ultimately protect cells against damage. After producing free radicals inside cytoplasm, the thioredoxin (Txr) signaling cascade is initiated in response to oxidative stress. Expression and functional activity of antioxidant enzymes such as catalase, glutathione peroxidase, glutathione reductase and thioredoxin reductase possibly also modifies the melanogenic pathway.[10,21] TrxR, component of Trx system, has also been reported to be involved in melanogenesis. Pigment-producing melanocytes cope with frequent oxidative stress in its physiological role in protecting the skin from the adverse effects of UV radiation. This is achieved through the activity of several endogenous antioxidant systems, including the thioredoxin antioxidant system, in which thioredoxin reductase plays an important role. Overall, this suggests that TrxR positively regulates melanocyte homeostasis and pigmentation during development and protects against DNA damage due to UVB and stress oxidative.[22] Function and expression of antioxidant enzyme activity such as glutathione reductase, glutathione peroxidase, and thioredoxin reductase, most likely modifying the melanosome pathway that associated with the occurrence of melasma.[10,22,23] Several studies have shown that increased expression of the TrxR gene and Trx plays a protective role against mammalian organs. There are also report that TrxR is highly expressed on the surface of keratinocytes and human melanocytes, which are the skin's first line of defense against free radicals produced in response to UV light.[12] As a conclusion, there is a relationship between TrxR levels and melasma.

In this study there were 15 subjects (50%) who had suffered from melasma for <5 years and 15 subjects (50%) who suffered from melasma for ⩾5 years. In this study the mean TrxR levels in the group of melasma subjects who suffering from <5 years was 11.4 ng/mL, while the average Trxr level in the group of melasma subjects suffering from ⩾5 years was 14.0 ng/mL. It has previously been shown that the thioredoxin system protects against injury skin due to UVB rays, as well as against peroxidative damage, and based on Saputra, Furqaani and Hikmawati's research shows that there is a relationship between long exposure to UV rays with the occurrence of melasma.[24] However, further research regarding TrxR levels based on the duration of suffering from melasma has never been done previously. In this study, the results of statistical analysis show that the TrxR mean levels were higher in melasma subjects suffering from ⩾5 years.

CONCLUSION

Based on the analysis of the data obtained from this study can be concluded that there was a significant relationship between TrxR level and melasma with p value <0.001, indicating that TrxR may have a role in the pathophysiology of melasma.

DECLARATIONS

The research has received approval from the Universitas Sumatera Utara and RSUP Haji Adam Malik Health Research and Ethics Committee with Number. All participants were informed about subject of the study.

CONSENT FOR PUBLICATION

The Authors agree to publication in Journal of Society Medicine.

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