



Antioxidant activity in melasma

Melazmada antioksidan aktivite

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Abstract

Background and Design: Melasma is a common, symmetric hypermelanosis characterized by irregular brown to gray-brown macules on the face. It is frequently associated with pregnancy and oral contraceptive consumption. Sunlight and genetic factors play major roles in the pathogenesis of melasma. Human skin exposed to ultraviolet light or environmental oxidizing pollutants become a preferred target of oxidative stress. Topical and oral antioxidants are used to treat melasma. To investigate serum antioxidant capacity in patients with melasma and relationship between antioxidant levels and melasma severity.

Materials and Methods: Forty-nine cases of melasma and 35 controls were included in the study. Each patient's skin pigmentation was assessed using the Melasma Area Severity Index (MASI) and mexameter reading. Serum trolox equivalent antioxidant capacity (TEAC), total antioxidant activity (TAOA), and ferric reducing power (FRAP) were evaluated in patients and controls by spectrophotometric method.

Results: TEAC levels were higher in patients than in controls ($p<0.00$). However, there was no statistically significant relationship of MASI with TEAC, TAOA and, FRAP.

Conclusion: According to our results, there is not a strong relationship between serum antioxidants and melasma severity. Therefore, we propose that antioxidant therapy may not be necessary in patients with melasma.

Keywords: Antioxidants, melasma, treatment

Öz

Amaç: Melazma sık görülen, yüzde kahverengiden gri-kahverengiyeye değişen düzensiz şekilli maküllerle karakterize simetrik bir hipermelanozistir. Çoğunlukla gebelik periyodu ve oral kontraseptif ilaç kullanımı ile ilişkilidir. Güneş ışığı ve genetik faktörler melazma patogeneğinde önemli rol oynamaktadır. Deri ultraviyole ışığı ve çevresel okside edici etmenlere maruz kalarak oksidatif stresin hedefi haline gelmektedir. Topikal ve oral antioksidanlar melazma tedavisinde kullanılmaktadır. Bu çalışmada amaç, melazmalı hastalarda serum antioksidan kapasitesini saptamak ve antioksidan düzeyleri ile melazma şiddeti arasındaki ilişkiyi belirlemektir.

Gereç ve Yöntem: Kırk dokuz melazma hastası ve 35 kontrol çalışmaya dahil edildi. Her hastada derideki pigmentasyon düzeyi Melazma Alan Şiddet İndeksi (MAŞİ) ve mexametre skoru kullanılarak değerlendirildi. Serum trolox eşdeğeri antioksidan kapasite (TEAC), total antioksidan aktivite (TAOA), demir iyonu indirgeyici antioksidan güç yöntemi (FRAP) düzeyleri spektrofotometrik olarak hasta ve kontrol grubunda değerlendirildi.

Bulgular: TEAC düzeyi hastalarda kontrol grubuna göre daha yüksek idi ($p<0,00$). Ancak, MAŞİ ile TEAC, TAOA ve FRAP arasında istatistiksel olarak anlamlı bir ilişki saptanmadı.

Sonuç: Araştırmamızda serum antioksidanları ile melazma şiddeti arasında ilişki saptanmadı. Bu sonuçlara göre, melazmalı hastalarda antioksidan tedavinin her zaman etkili olmayabileceği sonucuna varıldı.

Anahtar Kelimeler: Antioksidanlar, melazma, tedavi

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Introduction

Melasma is a common, symmetric hypermelanosis characterized by irregular brown to gray-brown macules on the face. It affects the quality of life of the patients¹. Sunlight and genetic factors play major roles in the pathogenesis of melasma. It is frequently associated with pregnancy, oral contraceptive usage, cosmetics, and phototoxic drugs^{1,2}.

Ultraviolet (UV) radiation is the major factor in the etiopathogenesis of melasma. Additionally, it has been found that reactive oxygen species (ROS) generated by UV can accelerate skin pigmentation¹⁻³.

There are a lot of treatment alternatives for melasma. Antioxidants, ROS scavengers and inhibitors of ROS production have been used in the treatment of melasma for the prevention of UV-induced melanogenesis^{1,3,7}. However, there has been no study on the antioxidant activity (AOA) of patients with melasma. To the best of our knowledge, this is the first study investigating antioxidant capacity of patients with melasma and the relationship between antioxidant capacity and the severity and type of melasma.

Materials and Methods

This study was conducted from October 2011 to October 2012 in Ege University Department of Dermatology and Venereology, Cosmetology Unit and Department of Biochemistry.

Forty-nine melasma patients and 35 healthy controls were enrolled in this study. The mean age of the melasma patients and controls was 38±5.81 (23-50) years and 39.83±9.52 (18-57) years, respectively.

All subjects gave written informed consent before participating in the study, which was approved by the Ethics Committee of Ege University, Faculty of Medicine (decision no: 11-7/19).

Those who were pregnant or lactating and who had used any topical or oral antioxidant medications for one month prior to the trial were excluded from the study.

The mexameter (Courage & Khazaka Electronic, Cologne, Germany) provides a reproducible estimate of the content of melanin and hemoglobin (erythema) and has an accuracy of ±5%. Values on the mexameter range from 1 to 1000, with zero representing white and 1000 representing black.

Melasma Area and Severity Index (MASI) score is calculated on the basis of the area of involvement, darkness of melasma, and homogeneity of pigmentation. Four areas on the face were evaluated: forehead, right malar region, left malar region, and chin, which represent 30%, 30%, 30%, and 10% of the face, respectively. MASI scores range from 0 to 24. MASI scores were grouped as group 1 for 5-10, group 2 for 11-20, and group 3 for >20.

Clinical appearance of melasma was classified according to Wood's light examination as epidermal, dermal, and mixed types. Photographs of all patients were taken. Serum trolox equivalent antioxidant capacity (TEAC), total AOA (TAOA), and ferric reducing antioxidant power (FRAP) were measured by spectrophotometric method.

Serum trolox equivalent antioxidant capacity measurement⁸

2,2'-azinobis 3-ethyl benz thiazoline sulfonate (ABTS) (7 mmol/L) and potassium persulphate (4.95 mmol/L) were mixed (1/1:v/v) and stored in room temperature at least for 12 h. before using. This reactive was diluted with phosphate buffer (1/25:v/v) until the absorbance value

reached up 1.0-1.5. 975 microliters of this working solution were mixed with 5-25 microliters serum and absorbances were read in 734 nm wavelengths in a spectrophotometer. Phosphate buffer and trolox were used as control and standard, respectively.

Determination of serum total antioxidant activity⁹

The solution of 1,1-diphenyl-2-picrylhydrazin (0.1 mM DPPH) was rapidly mixed well with serum sample (1/100; v/v). The decline in absorbance was recorded at 550 nm against an ethanol blank over a period of 20 minutes in 5 minutes intervals in a microplate reader. The decrease in absorbance corresponding to 100% radical scavenging was determined with a solution of pyrogallol in dimethyl sulfoxide (ca. 0.5%), which caused complete scavenging within seconds.

Determination of ferric reducing antioxidant power¹⁰

Mixing solution (10:1:1, v/v/v) of acetate buffer (10 mM, pH=3.6), 2,4,6 tripyridyl-s-triazine (10 mM) and FeCl₃ (20 mM) were added into serum sample and stored in room temperature for 30 min. Readings were done in 620 nm in a microplate reader.

The levels of TEAC, TAOA, and FRAP were compared between patients and controls.

The relationship of the type of melasma (epidermal, dermal, mixed types) with the levels of TEAC, TAOA, FRAP and melasma, MASI score, and mexameter readings (erythema, pigmentation) were investigated. The Kolmogorov-Smirnov, Shapiro-Wilk, Mann-Whitney U, NPar, and Kruskal-Wallis tests and Spearman's correlation coefficient were used for statistical analyses.

Results

The mean age of patients with melasma and controls was 37.76±5.81 (23-50) years and 39.83±9.52 (18-57) years, respectively. All patients and controls were female. The average disease duration was 6 years (1-18 years) (Table 1).

Table 2 shows TEAC, FRAP and TAOA levels in patients and controls. TEAC, FRAP, and TAOA levels ranged between 2 and 8 (7±1), 255 and 924 (605±128), and 10.616 and 38.482 (25.212±5.308), respectively. In control group, TEAC, FRAP, and TAOA levels ranged between 2 and 7 (5±1), 255 and 987 (669±191), and 10.616 and 41.136 (27.866±7.962), respectively.

Although there was a statistically significant difference in TEAC levels between patient and control groups (p=0.00), no statistically significant difference was found in FRAP (p=0.178) and TAOA (p=0.178) levels (Table 2).

MASI-group 1 was reported in 13 patients (26.5%), MASI-group 2 in 21 patients (42.9%), and MASI-group 3 was observed in 15 patients (30.6%). There was no statistically significant relationship of MASI scores with TEAC (p=0.407), FRAP (p=0.058), and TAOA (p=0.058) levels. According to Wood's lamp examination, 32 (65.3%) patients had

Table 1. Ages of the patients and duration of melasma

		n	Mean ± SD	Min.	Max.
Age	Patients	49	37.76±5,8	23	50
	Controls	35	39.83±9.5	18	57
Duration of melasma		49	6.12±4.5	1	18

SD: Standard deviation, Min.: Minimum, Max.: Maximum

epidermal, 2 (4.1%) dermal, and 15 patients had (30.6%) mixed type of melasma (Table 3). There was no statistically significant relationship between the type of melasma and TEAC ($p=0.583$), FRAP ($p=0.217$) and TAOA ($p=0.217$) levels.

Pigmentation and erythema levels assessed by mexameter ranged between 82 and 594 (280.70 ± 110.4) and 101 and 1029 (398.17 ± 114.3), respectively (Table 3).

There was no statistically significant relationship between the levels of erythema and pigmentation calculated by mexameter and TEAC, FRAP and TAOA levels.

Discussion

Although the relationship between oxidative stress and melasma is known, there are no published data with regards to a possible correlation between antioxidant capacity and melasma.

To the best of our knowledge, this is the first study investigating the relationship between melasma severity and antioxidant capacity.

In our study, it was found that there was no relationship between the severity of melasma and antioxidant capacity. On the other hand, only serum the TEAC level was higher in patients with melasma than in controls ($p=0.00$). This can be due to the existing high levels of natural antioxidants (uric acid, bilirubin etc.) in the subjects. In these cases, it was interesting that the high levels of antioxidant capacity had not any protective effect on the progression of melasma. This may be due to the other mechanisms playing a role in melasma pathogenesis other than oxidative stress.

UV radiation generates ROS and leads to oxidative stress¹¹. Increasing evidences in clinical studies suggest that oxidative stress is considered to be one of the main causative factors in the pathogenesis of melasma. This causes a cascade of erythema and inflammatory reactions, which may be considered as crucial factors affecting the pathogenesis of melasma^{1,11}. Both intra and extracellular antioxidant defense mechanisms exist to prevent tissue damage. This pathogenesis explains why antioxidants are used in the treatment of melasma. In the treatment of melasma, identification and elimination of causative factors, such as medications, cosmetics and sunlight, is important¹¹. The principles of therapy in melasma are to provide protection from UV radiation, retard the proliferation of melanocytes, inhibit the formation of melanin and melanosomes, and promote the degradation of melanin pigments by keratinocytes or melanophages¹.

There are a lot of treatment alternatives for melasma including topical application of hypopigmenting agents, chemical peels, lasers, and intense pulsed light. Oral or topical administration of antioxidants, such as vitamin A, C and E, has been reported to complement the standard regimens^{1,3,5,12-15}. Antioxidants, ROS scavengers and inhibitors of ROS production, have been used in the treatment of melasma for

the prevention of UV-induced melanogenesis^{1,3,7}.

We also investigated that TEAC, FRAP, and TAOA levels in epidermal, dermal and, mixed type of melasma in this study, but we did not find any relationship between antioxidant capacity and these melasma types. The few numbers of cases in dermal and epidermal groups can be responsible for this result. It is suggested that the determination of the indicative role of antioxidant capacity on the melasma to be dermal or epidermal is an interesting issue. Designing a similar study with larger samples would provide more significant data.

We did not find any correlation between these three parameters (TEAC, FRAP, TAOA). We believe that this was due to measurement methodology. Due to the difficulty in measuring each antioxidant component separately and interactions among antioxidants, methods have been developed to assess the total antioxidant capacity of serum or plasma. The 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), TEAC assay⁸, the oxygen radical absorbance capacity (ORAC) assay⁹, and the FRAP assay¹⁰ are commonly used and have been extensively evaluated. Although comparable results have been obtained with TEAC and ORAC assays, no correlation has been found between ORAC and TEAC values or between FRAP and TEAC values. We propose that TEAC levels can interfere with the generating of melasma, but antioxidant levels do not affect type of melasma.

While FRAP and ABTS show the ability of an antioxidant to transfer one electron to reduce any compound, such as metals, carbonyls, TEAC measures the ability of an antioxidant to quench free radicals. It is inevitable to find no correlation between these parameters. All these methods determine possible antioxidant potential and their levels are affected by the presence of antioxidant molecules such as, bilirubin, uric acid, vitamin E, vitamin C, and foods having high amount of polyphenolic compounds. Since *in vivo* antioxidant molecules were

Table 3. Melasma types and Melasma Area and Severity Index scores of the patients

Type of melasma	n	%
Epidermal	32	65.3
Dermal	2	4.1
Mixt	15	30.6
MASI	n	%
5-10	13	26.5
11-20	21	42.9
>20	15	30.6
Mexameter	n	Mean ± SD
Pigmentation	35	286.86±110.2
Erythema	35	379.17±94.2

SD: Standard deviation, MASI: Melasma Area and Severity Index

Table 2. The levels of antioxidant parameters in controls and patient with melasma (min.-max. ± SD)

	TEAC (µmol/mL trolox eq)	FRAP (µmol/L FeSO ₄ eq)	TAOA (µmol/mL trolox eq)
Control (n=37)	2-7 (5±1)	255-987 (669±191)	10616-41136 (27866±7962)
Patient (n=50)	2-8 (7±1)*	255-924 (605±128)	10.616-38.482 (25212±5308)

* $p=0.001$, TEAC: Trolox equivalent antioxidant capacity, FRAP: Ferric reducing antioxidant power, TAOA: total antioxidant activity, FeSO₄: Iron (II) sulfate
min.: Minimum, max.: Maximum

not determined, we cannot make a clear suggestion on the correlation between the severity of the disease and antioxidant capacity of subjects.

Study Limitations

The lack of measurements of the endogenous antioxidants, such as uric acid and bilirubin, and the relatively small number of cases are the limitations of the study.

Conclusion

As a result, our findings suggest that plasma antioxidant capacity is not primarily responsible for different types of melasma and its severity.

Statements

Plasma antioxidant capacity is not primarily responsible in melasma pathogenesis. Oral and topical antioxidants may not be effective in melasma treatment.

Ethics

Ethics Committee Approval: The study were approved by Ege University, Faculty of Medicine, Ethics Committee decision no.: 11-7/19.

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship contributions

Surgical and Medical Practices: İ.E., T.Ö., Concept: İ.E., Design: İ.E., İ.Ü., Data Collection or Processing: İ.E., T.Ö., Analysis or Interpretations: Y.A., E.Y.S., Literature Search: İ.E., T.Ö., Writing: İ.E., T.Ö., İ.Ü.

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