Original Article

Increased Oxidative Stress and Imbalance Dynamic Thiol–Disulfide Homeostasis in Rosacea

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Abstract

Background: Rosacea is a chronic progressive inflammatory disease and characterized by facial erythema, telangiectasias, papules, and pustules. The disease is more common in women than in men while affecting 2%–10% of the population. Though the pathogenesis of rosacea is not fully understood, oxidative stress is one of the asserted pathogenic factors. In this study, we purposed to assess the oxidative stress and thiol–disulfide homeostasis (TDH) in rosacea disease. **Materials and Methods:** Forty patients with rosacea and 40 healthy people as a control group, both in ages of 18–70 years, with the same demographic characteristics were included, who were applied to the Istanbul Training and Research Hospital Dermatology Clinic. Serum total antioxidant status (TAS), total oxidant status (TOS), total thiol (TT), and native thiol (NT) levels were evaluated by using the automated and spectrophotometric method according to Erel and Neselioglu. Oxidative stress index (OSI), disulfide (DIS) levels, and NT/TT, DIS/TT and DIS/NT percentages were calculated mathematically. **Results:** TAS, TT, and NT levels were decreased in rosacea patients compared with the healthy group, whereas TOS, OSI, and DIS levels were increased. Additionally, although DIS/TT and DIS/NT percentages were higher in patients, NT/TT ratio was lower than the healthy group, and these findings were statistically significant ($p < 0.001$). **Conclusion:** The results showed that oxidative stress levels were increased in rosacea patients and TDH shifted toward DIS formation. It has been thought that oxidative stress is a parameter that may be utilized in the clinical evaluation of the disease.

Keywords: Disulfide, inflammation, oxidative stress, rosacea, skin disease, thiol

Introduction

Rosacea is a chronic inflammatory skin disease that is thought to affect 2%–10% of the population and is more common in women than in men.[1] The disease features are flushing, nontransient erythema, papules/pustules, telangiectasia, and phymatous changes.[2] Secondary symptoms such as burning or stinging sensation, plaque, a dry appearance of the skin, edema, ocular manifestations, and phymatous changes could sight in patients.[3] There are four different clinical subtypes related to the disease according to the patterns of physical symptoms.[4] These subtypes are phymatous rosacea, erythematotelangiectatic rosacea, papulopustular rosacea, and ocular rosacea.[5]

Although the pathogenesis of rosacea is not exactly comprehended, some pathogenic factors have been asserted to be. In addition to these, environmental triggers such as ultraviolet irradiation, temperature changes, alcohol, physical activity, foods, and chemicals are also thought to be related.[6] The disease includes local inflammatory responses in the skin against cutaneous microorganisms such as *Demodex folliculorum* and *Demodex brevis* and changes in the lymphatic vascular systems.[7] Recent studies have shown that rosacea, which is an inflammatory disease, is relevant to

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the generation of reactive oxygen species (ROS) released by inflammatory cells.[8]

The imbalance between the number of present antioxidant molecules and the overproduction of ROS is defined as oxidative stress.[9] The oxidation of proteins and lipids occurs as a result of the deactivation of natural defenses by oxidative stress. As a result of the changes in the lipid balance, inflammation occurs with the release of other inflammatory mediators such as cytokines.[10] Dynamic thiol–disulfide homeostasis (TDH), which is the ratio between the thiol and disulfide (DIS) parameters, is another parameter that indicates the balance of oxidant–antioxidant status.[11] The dynamic thiol-disulfide balance could be maintained by the reduction of thiol groups converted to DIS structures back to thiol groups.[12] This homeostasis is involved in important functions such as the regulation of enzymatic activity, apoptosis, signal transduction, and antioxidant protection.[13] The disruption of dynamic TDH and also oxidative stress causes many diseases such as asthma,[14] inflammatory diseases, cardiovascular diseases,[15] diabetes,[16] rheumatoid arthritis,[17] kidney diseases,[18] and cancer.[19] As in many inflammatory diseases, oxidative stress is thought to be one of the important factors for the pathophysiology of rosacea disease.[20] We purposed to investigate and evaluate the levels of oxidative stress that are important to understand the pathogenesis of the disease in patients with rosacea.

Materials and Methods

Design of study

The persons were selected from those diagnosed with rosacea according to the criteria of the United States National Rosacea Society,[21] who were applied to the Istanbul Training and Research Hospital Dermatology Clinic. The Fitzpatrick classification was utilized to identify the skin types of patients.[22] The number of volunteers was calculated as a minimum of 80 volunteers by power analysis at the $\alpha = 0.05$ significance level to obtain 80% power in the study. Forty patients with rosacea ages of 18–70 years and 40 healthy volunteers as a control group with the same demographic characteristics were included, who stated that they would participate in the study by reading and signing the consent form. The patients or persons having dermatological or systemic; acute or chronic inflammatory disease affecting oxidative stress parameters, kidney disease, liver disease, neurodegenerative disease, cancer, the use of drugs that have an effect on the cardiovascular system, and smokers would be excluded. It was approved by the decision of the Clinical Research Ethics Committee of Hamidiye University of Health Sciences, numbered 22/167, and dated 25/03/2022.

Blood sample

Blood samples of volunteers were taken into sterile gel tubes with a clot activator and were centrifuged at 3000× g for 10 min. Until the specified number of individuals was reached and biochemical analysis was performed, separated serum samples were stored at −80°C.

Measurement of oxidative stress levels

Total antioxidant status (TAS) was measured according to the Erel's fully automatic photometric method.[23] The presence of antioxidants is reduced to the blue–green color of the 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$; Sigma, St. Louis, USA) radical that the main principle of this test. The color change resulting from the reaction was measured with a multimode plate reader at 600 nm wavelength. Ascorbic acid was used as a standard in the measurement. Results were expressed as mmol ascorbic acid equivalent per liter (mmol Ascorbic Acid Eq./L).

Total oxidant status (TOS) is based on the principle that xylenol orange forms a colored complex by adding a ferric ion-containing reagent in an acidic condition, according to the photometric method of Erel.[24] The Fe^{2+}-o-dianisidine complex is converted to Fe^{3+} ion with the oxidants in the sample by oxidizing. Color intensity was measured spectrophotometrically at a wavelength of 571/658 nm in relation to the total amount of oxidant molecules present. H_2O_2 (Sigma Aldrich, Germany) was used for calibration, and results were expressed as H_2O_2 equivalent per liter (μmol H_2O_2 Eq./L). Oxidative stress index (OSI) was obtained by dividing serum TOS and TAS levels.

Measurement of TDH

Serum TDH was measured by the photometric method according to Erel and Neselioglu.[25] Free thiol groups from reducible DIS bonds are formed by sodium borohydride, which in excess is removed with formaldehyde, followed by a reaction with 5,5′-dithiobis-(2-nitrobenzoic) acid (DTNB; Sigma, St. Louis, USA) to reduce thiol groups. In this way, the total thiol (TT) containing the reduced and native thiol (NT) groups were determined. Half the difference between TT and NT groups for dynamic DIS content, DIS/TT, DIS/NT, and NT/TT percentages were calculated with mathematical formulas.

Statistical analysis

Categorical variables were expressed as numbers and percentages, and numerical variables were also as mean ± standard deviation. The Mann–Whitney *U* test and the Student's *t*-test were utilized for the comparison of two independent groups. The Statistical Package for Social Sciences (SPSS, New York, USA) program 25.0 version was used for all statistical analysis.

Results

In this study, the groups were expressed in terms of age (39 ± 10.10 years), gender (68 female + 32 male), and body mass index (25 ± 2.95), and there was no statistically

significant difference between the groups. The participants' demographic characteristics were given in Table 1.

According to the Fitzpatrick classification of skin types, skin type 2 was the most common in the individuals participating in our study. However, the erythematotelangiectatic subtype was seen in the majority of rosacea patients, followed by phymatous and papulopustular in Table 2.

Although TOS and OSI levels were higher in rosacea patients than in the control group with increased oxidative stress, TAS levels were found to be lower. Dynamic TDH as an oxidative stress parameter is given in Table 3. The DIS levels, which indicate oxidative damage, were raised in patients compared with controls. Additionally, NT and TT levels, which are indicators of antioxidant capacity, were also statistically significantly lower than the control group ($p < 0.001$).

Discussion

Rosacea, which is a progressive inflammatory disease, is characterized by facial erythema, pustules, papules, and telangiectasias. The disease occurs between the ages of 30–50 and shows similar incidence rates in men and women additionally.[26] It is assumed that rosacea is associated with different dysregulated nervous, immune, vascular systems, and the disease could be triggered by microbes, temperature changes, ultraviolet radiation, nutrition, hormones, and psychosocial stress, inducing an enhanced innate immune response.[27] Oxidative stress is the formation of excess ROS at levels higher than the body's antioxidant capacity. Recent studies have shown that oxidative stress is related to oxidative tissue damage, vascular changes, and inflammation in rosacea and has an important role in the pathophysiology of the disease.[28] Thiol levels are one of the parameters that give information about oxidative stress and also are valuable for understanding the underlying causes of the rosacea disease. In this study, the dynamic TDH and oxidative stress levels of rosacea patients were purposed to evaluate.

Recent studies have suggested that oxidative stress conduces to the pathophysiology of skin diseases, which is more sensitive to damage caused by ROS than other tissues. The presence of ROS destroys collagen and inactivates tissue inhibitors of matrix metalloproteinases.[29] In the study by Tisma *et al.*, serum total antioxidant capacity in rosacea patients was lower than in the healthy group, whereas serum peroxide levels were higher in the patients.[30] Erdogan *et al.* have shown that serum TAS levels decreased in rosacea patients compared with healthy volunteers as serum oxidant levels increased.[31] This study has shown that antioxidant levels decreased depending on the increase in ROS level in the patients, and TOS and OSI increased compared with healthy volunteers. Our findings were consistent with the literature.

Although NT simply means nonreduced thiol, TT consists of both reduced and nonreduced thiols, which include both intracellular and extracellular thiols.[32] Thiols contain sulfhydryl residues as the class of organic sulfur derivatives. It is entrusted with antioxidant defense and also has many functions in biological systems. The level of DIS bonds is an early indicator of protein oxidation caused by ROS.[33] Thiol groups are oxidized to form DIS bonds with the presence of oxidants, thereby plasma thiol levels were decreased. Dynamic TDH is defined as the reduction of formed DIS bonds back to reduced thiol groups.[34] The shift of TDH to DIS formation causes pathologies in the structure, and functions of many organs so result in the emergence of various diseases.[35,36]

There are few studies investigating dynamic TDH in rosacea patients in the literature. Sener *et al.* found that serum DIS levels were higher in rosacea patients compared to the control group. It was determined that the dynamic TDH shifted toward DIS formation with the rosacea disease. Additionally, DIS/TT and DIS/NT ratios were found to be higher in patients compared with the healthy control group, whereas NT/TT ratios were found to be lower.[37] TDH has been investigated in an inflammatory skin disease named lichen planus. TT and NT levels were compared with the healthy control group and found to be lower in these patients.[38] Aksoy and Kirmit examined the levels of thiol and DIS in psoriasis, which is a chronic inflammatory disease of the skin. In this study, serum TT and NT levels of psoriasis patients were detected to be lower compared with the healthy control group, whereas serum DIS levels were found to be higher.[39] Pektas *et al.* found DIS levels to be higher in psoriasis patients than in the control group, whereas TT and NT levels were lower in patients than the control group. The percents of DIS/NT and DIS/TT were found to be lower compared with

Table 1: Demographic characteristics of the participants

		Control ($n = 50$)	Patients ($n = 50$)
Age (years)		39.64 ± 10.10	38.98 ± 10.25
Gender (n [%])	Male	10 (25)	11 (28.95)
	Female	30 (75)	27 (71.05)
BMI (kg/m^2)		24.35 ± 2.72	24.94 ± 3.16

BMI = body mass index (kg/m^2)

Table 2: Skin types and subtypes of rosacea patients

	Types	Patients (n [%])
Fitzpatrick skin type	2	35 (70)
	3	10 (20)
	4	5 (10)
Rosacea type	Erythematotelangiectatic	32 (64)
	Papulopustular	7 (14)
	Phymatous	11 (22)

Table 3: According to the control, comparing oxidative stress parameters in rosacea patients

	Control ($n = 50$)	Patients ($n = 50$)	p
TAS (mmol Trolox Eq./L)	1.20 (0.83–1.89)	0.82 (0.62–1.32)	0.001[a]
TOS (μmol H_2O_2 Eq./L)	11.01 (9.34–13.95)	13.95 (10.35–18.52)	0.001[a]
OSI (arbitrary units)	9.69 ± 1.96	16.47 ± 3.34	0.001[b]
TT (μM)	491.83 (378.65–576.47)	386.88 (247.88–449.13)	0.001[a]
NT (μM)	339.30 ± 65.50	219.18 ± 61.36	0.001[b]
DIS (μM)	73.37 ± 31.10	81.16 ± 35.80	0.248[b]
DIS/TT (%)	15.04 ± 5.96	20,95 ± 8.63	0.001[b]
DIS/NT (%)	19.93 (3.46–67.99)	36.81 (2.91–130.67)	0.001[a]
NT/TT (%)	69.91 ± 11.93	58.09 ± 17.27	0.001[b]

Eq. = equivalent

[a]Mann–Whitney U test ($p < 0.05$, statistical significance)

[b]Independent T test

the healthy control. However, the percents of NT/TT were higher in the patient than in the healthy group.[40] In this study, TT and NT levels were decreased in rosacea patients than in the healthy control group, whereas DIS levels were increased. DIS/NT and DIS/TT percentages were higher in rosacea patients compared with controls, whereas NT/TT ratios were lower. TDH parameters are similar to studies in patients with rosacea and other inflammatory skin diseases in the literature.

The number of patients in the study was not sufficient and the study participants might not be representative of patients from the general population. In addition, clinical scoring of the disease is among the limitations of the study because of a semi-quantitative evaluation method.

Conclusion

We investigated OSI and dynamic TDH, which are the oxidative stress biomarkers that could be utilized in understanding the pathogenesis of rosacea. The results have been shown that TOS, OSI, and DIS levels increased in patients with rosacea, whereas the levels of TAS, TT, and NT, which are involved in antioxidant defense, decreased. It has been observed that TDH shifts toward DIS formation in patients with rosacea. It is thought that our results could be a guide in understanding the factors that cause the disease and in the development of current treatments.

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Conflicts of interest

There are no conflicts of interest.

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