Evaluation of Ocular and Systemic Oxidative Stress Markers in Ocular Rosacea Patients

Nilufer Yesilirmak, Neslihan Bukan, Busra Kurt, Sema Yuzbasioglu, Min Zhao, Daniela Rodrigues-Braz, Akin Aktas, Francine Behar-Cohen, Jean-Louis Bourges

Evaluation of Ocular and Systemic Oxidative Stress Markers in Ocular Rosacea Patients

Nilufer Yesilirmak,[1–4] Neslihan Bukan,[4] Busra Kurt,[3] Sema Yuzbasioglu,[3] Min Zhao,[1] Daniela Rodrigues-Braz,[1] Akin Aktas,[5] Francine Behar-Cohen,[1,2] and Jean-Louis Bourges[1,2]

[1]Centre de Recherche des Cordeliers, INSERM, Université Paris Cité, Sorbonne Université, Physiopathology of ocular diseases: Therapeutic innovations, Paris, France
[2]Ophthalmopole Cochin University Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France
[3]Department of Ophthalmology, Ankara Yildirim Beyazit University, Ankara City Hospital, Ankara, Turkey
[4]Department of Medical Biochemistry, University of Gazi, Ankara, Turkey
[5]Department of Dermatology, Ankara Yildirim Beyazit University, Ankara City Hospital, Ankara, Turkey

Correspondence: Nilufer Yesilirmak, Centre de Recherche des Cordeliers, INSERM, Université Paris Cité, Sorbonne Université, Physiopathology of ocular diseases: Therapeutic innovations, Paris, France; dryesilirmak@gmail.com.

Received: July 19, 2023
Accepted: September 21, 2023
Published: October 16, 2023

Citation: Yesilirmak N, Bukan N, Kurt B, et al. Evaluation of ocular and systemic oxidative stress markers in ocular rosacea patients. *Invest Ophthalmol Vis Sci.* 2023;64(13):22. https://doi.org/10.1167/iovs.64.13.22

Purpose. To investigate oxidative stress markers in tears and serum of patients with ocular rosacea and to examine their association with both ocular surface parameters and cutaneous rosacea subtypes.

Methods. This prospective study includes rosacea patients with ocular involvement and healthy controls. We performed ophthalmological examination of all participants and collected tear breakup time (TBUT), Schirmer, Meibomian gland dysfunction (MGD) and Ocular Surface Disease Index (OSDI) scores. We quantified the total antioxidant status (TAS), total oxidant status (TOS), and arylesterase (ARE) levels from tear and serum samples, and calculated the oxidative stress index (OSI). We also classified patients into phymatous, erythematotelangiectatic, papulopustular subtypes.

Results. We included 90 ocular rosacea patients and 30 healthy controls. Oxidative stress (TOS, OSI) levels were significantly higher ($P < 0.01$) and antioxidant levels (TAS, ARE) were significantly lower ($P < 0.01$) in both tear and serum samples of ocular rosacea patients as compared to controls. We found a significant positive correlation between the tear and serum values regarding oxidative stress parameters ($P < 0.05$). Besides, OSI was negatively correlated with TBUT and positively correlated with MGD score (meiboscore) and OSDI ($P < 0.05$). The Schirmer score was not correlated with OSI. No difference was found between the cutaneous subtypes with respect to TAS, TOS, ARE, and OSI results.

Conclusions. In this study, we identified oxidative stress markers in the serum and tears of ocular rosacea patients and showed their correlation with clinical signs of MGD, suggesting that oxidative stress contributes to ocular rosacea pathogenesis and that oxidative stress could be an indicator of MGD severity.

Keywords: ocular surface, ocular rosacea, tear oxidative stress status, serum oxidative stress status, meibomian gland dysfunction

Rosacea is a common disease, featuring a chronic facial inflammation characterized by flushing, papules, pustules, telangiectasia, and ocular manifestations. It affects approximately 10% of the population, with those with fair sun-sensitive skin being at a greater risk.[1] Ocular rosacea represents up to 50% of the total rosacea population. It is characterized by lid margin telangiectasia, blepharitis, meibomian gland dysfunction, interpalpebral conjunctival injection, spade-shaped corneal infiltrates, scleritis, or sclerokeratitis.[2] In severe cases, corneal damage, such as corneal neovascularization, corneal thinning, and perforation, can compromise both corneal transparency and integrity, leading to potential severe visual impairment.[3] Although the etiopathology of rosacea is not fully understood, it has been reported that innate and adaptive immune system dysregulation, vascular changes, genetic predisposition, ultraviolet radiation, demodex proliferation, microbial stimuli, and oxidative stress damages play a role in its pathophysiology.[4–6]

Oxidative stress damage results from an imbalance between the production of reactive oxygen species (ROS) and the ability of the body to neutralize and detoxify them.[7] ROS are highly reactive molecules that can alter proteins, lipids, mitochondria, and DNA, leading to irreversible cell damage and subsequent inflammation.[8] Previous studies showed that patients with rosacea display skin markers of oxidative stress such as higher ROS, lipid peroxidation products, and decreased levels of antioxidant enzymes including superoxide dismutase, paraoxonase, and arylesterase (ARE).[9–12] But whether it is subsequent to ocular rosacea or it contributes to initiate the disease is unclear.

In recent years, global index of the total oxidative stress status of an individual is being calculated by measuring total antioxidant status (TAS) and total oxidant status (TOS) with

a newly developed method.[13,14] In addition, the oxidative stress index (OSI), obtained from the ratio of TOS and TAS levels, is used to provide a comprehensive measure of oxidative stress in the organism.[13] Two studies have investigated the total oxidant/antioxidant status in the serum of patients with cutaneous rosacea.[15,16] But, according to our knowledge, none of the previous studies have evaluated oxidative stress from serum or tear samples of ocular rosacea patients. Furthermore, no study has yet investigated the relationship between tear oxidative stress status and ocular surface clinical characteristics in ocular rosacea patients. In this prospective study, we have investigated tear and serum TOS, TAS, OSI, and ARE levels and analyzed the relationship between these oxidative stress levels and patient's skin phenotype (cutaneous rosacea subtypes) and their impact on ocular surface clinical parameters.

Methods

Study Population

This prospective study included 90 rosacea patients diagnosed with cutaneous rosacea by a senior dermatologist (A.A.) and confirmed as having rosacea-related ocular involvement by a senior ophthalmologist (N.Y.) at Ankara City Hospital (between July–December 2021). The group of rosacea patients was age-matched with 30 healthy controls. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Ankara City Hospital (no. E2-21-1156). An informed consent was obtained from all participants after explanation of the nature and possible consequences of the study.

Inclusion and Exclusion Criteria

The patients who had been diagnosed with cutaneous and ocular rosacea according to the ROSacea COnsensus (ROSCO) panel classification and had not yet used any medication were included in this study.[17] Patients with ocular rosacea–related[17] lid margin telangiectasia, blepharitis, blepharoconjunctivitis, and meibomian gland dysfunction (MGD) were included (a representative pictures of an ocular rosacea patient are given in Fig. 1). Patients with an history of any other ocular or systemic disease (including the conditions that can cause MGD and dry eye other than ocular rosacea), severe ocular rosacea (affecting cornea), pregnancy, ocular or systemic medication (including supplements), smoking and alcohol consumption were excluded from the study.

Data Collection

Patient's demographics, ophthalmological examination features, tear breakup time (TBUT) measurements, Schirmer test score, MGD and Ocular Surface Disease Index (OSDI) scores were recorded. The TBUT was evaluated after 5 µL of fluorescein was placed on the conjunctivae. After blinking several times, the time between the last blink and the appearance of the first black spot on the cornea was recorded.[18] The Schirmer test was performed by placing a Schirmer strip in the lateral canthus of the inferior lid margin without topical anesthetics. After waiting for five minutes with the eyes closed, the wetting amount of the strips was obtained in millimeters.[19] The MGD score (meiboscore) was obtained by taking infrared meibography images of the inferior eyelids with the Sirius topography device (Sirius; CSO, Florence, Italy) and grading the loss rate in the glands on a scale of 0 to 4 (Grade 0: <10% loss; Grade 1: 10%–25% loss; Grade 2: 25%–50% loss; Grade 3: 50%–75% loss; Grade 4: >75% loss).[20] Representative meibography images showing patients' meibomian gland loss rates in Figure 2. For OSDI scores, the 12-item OSDI questionnaire with scores ranging from 0 to 100 was used.[21]

Sample Collection

Tear Collection. Tear samples were collected on Schirmer's strips within morning hours by preventing tear overflow. The strips were placed in the lower pouch of the eye for five minutes without anesthesia. Patient's tear samples were collected from the right eye only. A practical Schirmer's strip impregnation length of 10 to 15 mm was required to proceed further for biochemical analysis, compliant with our preliminary tests and literature findings.[22] To collect an equal volume of fluid from all patients, strips were shortened at the 15 mm mark after the Schirmer test

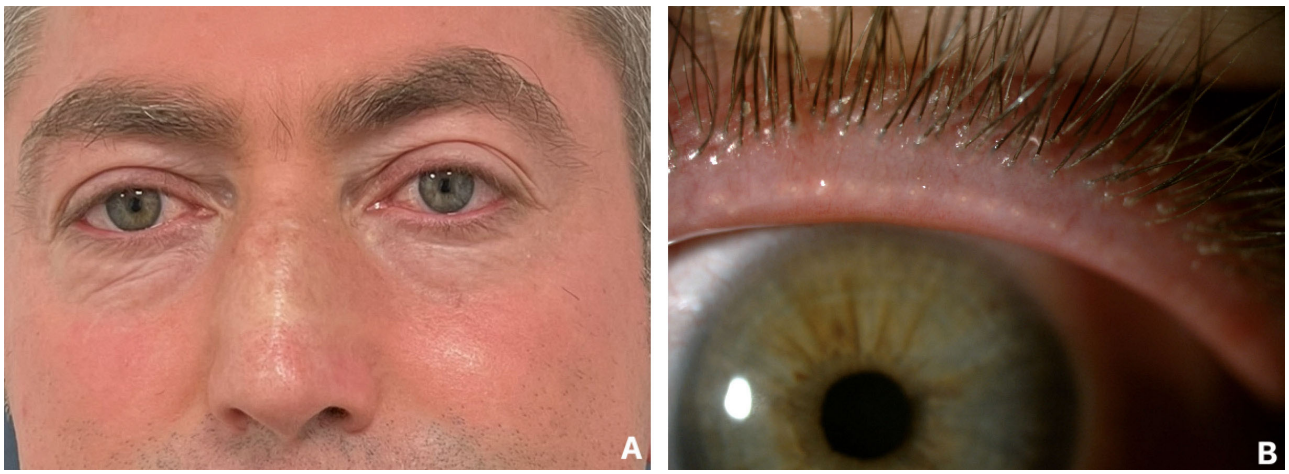

Figure 1. Representative pictures of an ocular rosacea patient. (**A**) Photography showing the patient' face with erythematotelangiectatic subtype. (**B**) Anterior segment photography showing the patients' ocular findings (blepharitis, obstructed meibomian glands and lid margin telangiectasia).

Investigative Ophthalmology & Visual Science

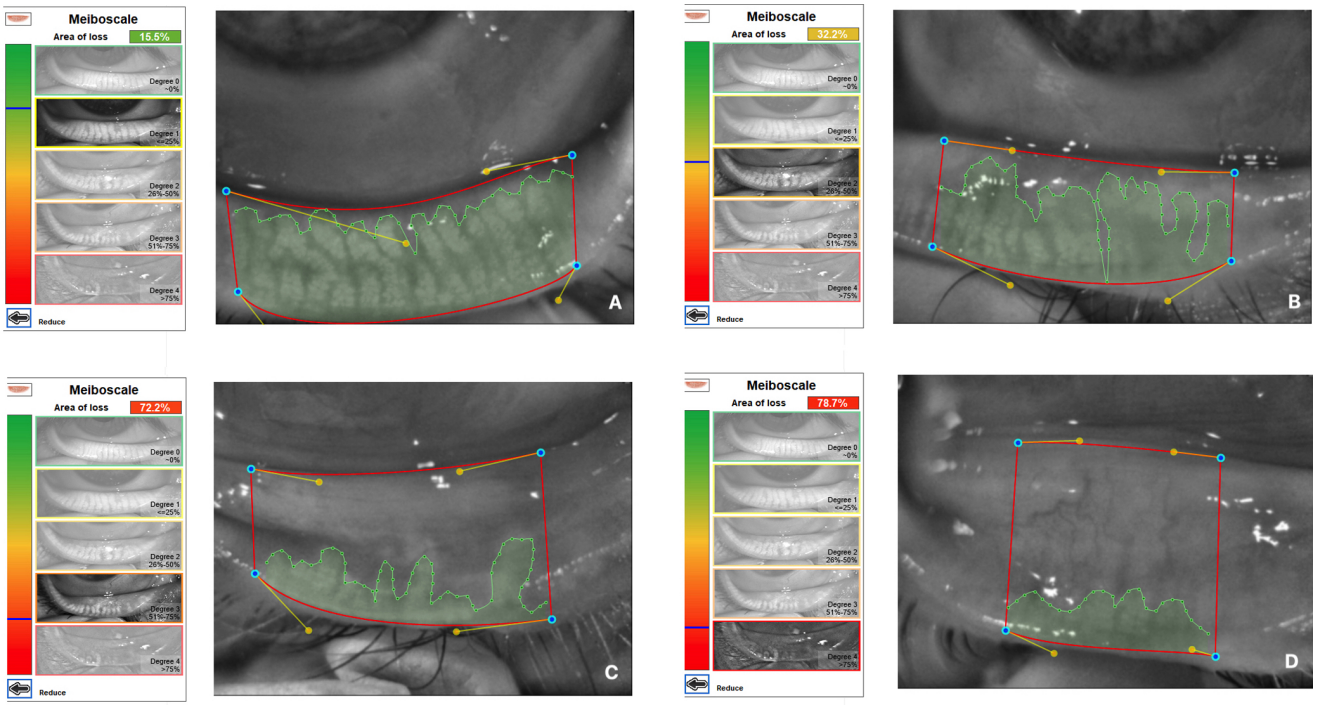

Figure 2. Representative meibography images showing patients' meibomian gland loss rates. (**A**) A patient with a gland loss rate 15.5% (Grade 1). (**B**) A patient with a gland loss rate 32.2% (Grade 2). (**C**) A patient with a gland loss rate 72.2% (Grade 3). (**D**) A patient with a gland loss rate 78.7% (Grade 4).

was completed. Then strips were placed in Eppendorf tubes, and the tubes were filled with previously cooled 200 µL PBS to prevent evaporation. In patients with poor tearing, strips could be harvested up to 10 minutes after the Schirmer test score was evaluated. Each sample was stored at −80°C immediately after collection until the day of biochemical analysis.

Serum Collection. Blood samples were collected after an overnight fast of eight to 10 hours. Blood samples were collected in clot activator tubes and immediately spun in a centrifuge at 4000 rpm for 10 minutes. Ultimately, serum samples were stored in 1.5 mL Eppendorf tubes at –80°C until the day of biochemical analysis.

Biochemical Analysis

All tear samples were spun in a centrifuge at 4000 rpm for 10 minutes just before analysis and the liquid component was transferred to a new Eppendorf tube. All tear and serum samples were analyzed simultaneously. Oxidative stress parameters (TAS, TOS, and ARE) were measured by using commercial kits (Rel Assay Kit Diagnostics; Rel Assay Diagnostics, Şehitkamil/Gaziantep, Turkey) and a fully automated spectrophotometer (Roche/Hitachi Modular; Roche, Basel, Switzerland).

TAS Measurements. The TAS levels of the tear and serum were determined using an automated colorimetric measurement method based on bleaching of the characteristic color of 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical cation.[13] Trolox, a water-soluble analogue of vitamin E, was used as a calibrator. This method determines the antioxidant effect of the sample against strong free radical reactions initiated by the hydroxyl radical product. The results were expressed in mmol Trolox equivalents/L.

TOS Measurements. The tear and serum TOS levels were determined using an automated colorimetric measurement method.[14] Oxidants present in the sample oxidized ferrous o-dianisidine complexes into ferric ions. Glycerol molecules enhanced the oxidation reaction. The ferric ions formed a colored complex with xylenol orange an acidic medium. Therefore the color intensity, measured spectrophotometrically, was related to the total number of oxidant molecules present in the sample. Hydrogen peroxide (Sigma-Aldrich Corp., St. Louis, MO, USA) was used for calibration, and the results were expressed in micromolar hydrogen peroxide equivalent per liter (µmol H_2O_2 equiv/L).

OSI Calculations. The ratio of TOS/TAS level was rendered the OSI.[14] Results were expressed as an "arbitrary unit" and calculated according to the following formula: OSI = TOS (µmol H_2O_2 equiv/L)/TAS (mmol Trolox equiv/L) × 10.

ARE Measurements. The ARE activity of both tears and serum were measured using phenylacetate as the substrate.[23] Enzymatic activity was calculated from the molar absorptivity coefficient (1310 $M^{-1} \bullet cm^{-1}$) of the produced phenol. One unit of ARE activity was defined as 1 µmol phenol generated/min under the above-defined assay conditions and expressed as kU/L serum.

Statistical Analysis

SPSS Statistics 22.0 was used to calculate means and standard deviations (SD) for all variables. Comparison plots were made using the GraphPad Prism 5 program (GraphPad Software, San Diego, CA, USA). Global data were reported as mean ± SD. The Shapiro-Wilk test was used to test normality of values. For normally distributed values, the independent sample *t*-test and one-way ANOVA were used to compare

Investigative Ophthalmology & Visual Science

the groups. The Mann-Whitney U and Kruskal Wallis tests were used to compare groups when related values were not normally distributed. For correlation analysis, the Pearson test was carried out for normally distributed values whereas the Spearman test was carried out for not-normally distributed values. A P value < 0.05 was considered as statistically significant.

Results

This study included 90 ocular rosacea patients (24 males, 66 females) who met the inclusion and exclusion criteria and 30 healthy individuals as "healthy controls" (10 males, 20 females). Mean age for rosacea patients was 51.72 ± 9.65 years, and 48.20 ± 10.03 years for healthy controls.

Table 1. Comparison of Demographic Characteristics and Ocular Surface Parameters Between Patients and Controls

	Patients (Mean ± SD) (n = 90)	Controls (Mean ± SD) (n = 30)	P Value
Age (y)	51.72 ± 9.65	48.20 ± 10.03	0.10
Gender (male/female)			0.32
Male	24	10	
Female	66	20	
TBUT score (sec)	5.54 ± 3.78	14.32 ± 3.99	<0.001
Schirmer score (mm)	15.57 ± 5.07	16.64 ± 5.73	0.34
Meiboscore (0–4)	2.39 ± 1.01	0.77 ± 0.76	<0.001
OSDI score (0–100)	41.92 ± 16.56	6.24 ± 3.73	<0.001

Table 2. Comparison of Oxidative Stress Parameters Between Patients and Controls

	Patients (Mean ± SD) (n = 90)	Controls (Mean ± SD) (n = 30)	P Value
TAS serum	1.12 ± 0.22	1.43 ± 0.22	**<0.001**
TOS serum	7.90 ± 2.39	5.57 ± 1.29	**<0.001**
ARE serum	321.37 ± 16.09	343.10 ± 14.38	**<0.001**
OSI serum	0.73 ± 0.26	0.40 ± 0.11	**<0.001**
TAS tear	0.21 ± 0.07	0.36 ± 0.13	**<0.001**
TOS tear	2.20 ± 0.61	1.49 ± 0.31	**<0.001**
ARE tear	263.34 ± 7.02	282.30 ± 11.66	**<0.001**
OSI tear	1.10 ± 0.43	0.46 ± 0.16	**<0.001**

Statistically significant difference was found between the groups.

There was no statistically significant difference between patients and controls for age and gender (P = 0.10 and P = 0.32) (Table 1). All of the patients featured mild-to-moderate ocular rosacea, including varying degrees of lid margin telangiectasia, blepharitis, blepharoconjunctivitis, and meibomian gland dysfunction.

Table 2 and Figure 3 show the comparison of TAS, TOS, OSI, and ARE values between patients and controls. Tear and serum TOS and OSI values (indicating oxidative stress) were significantly higher ($P < 0.01$), whereas TAS and ARE values (indicating antioxidant status) were significantly lower ($P < 0.01$) in ocular rosacea patients compared to healthy controls.

Figure 4 shows the correlation of oxidative stress parameters (TAS, TOS, OSI, and ARE) between tear and serum

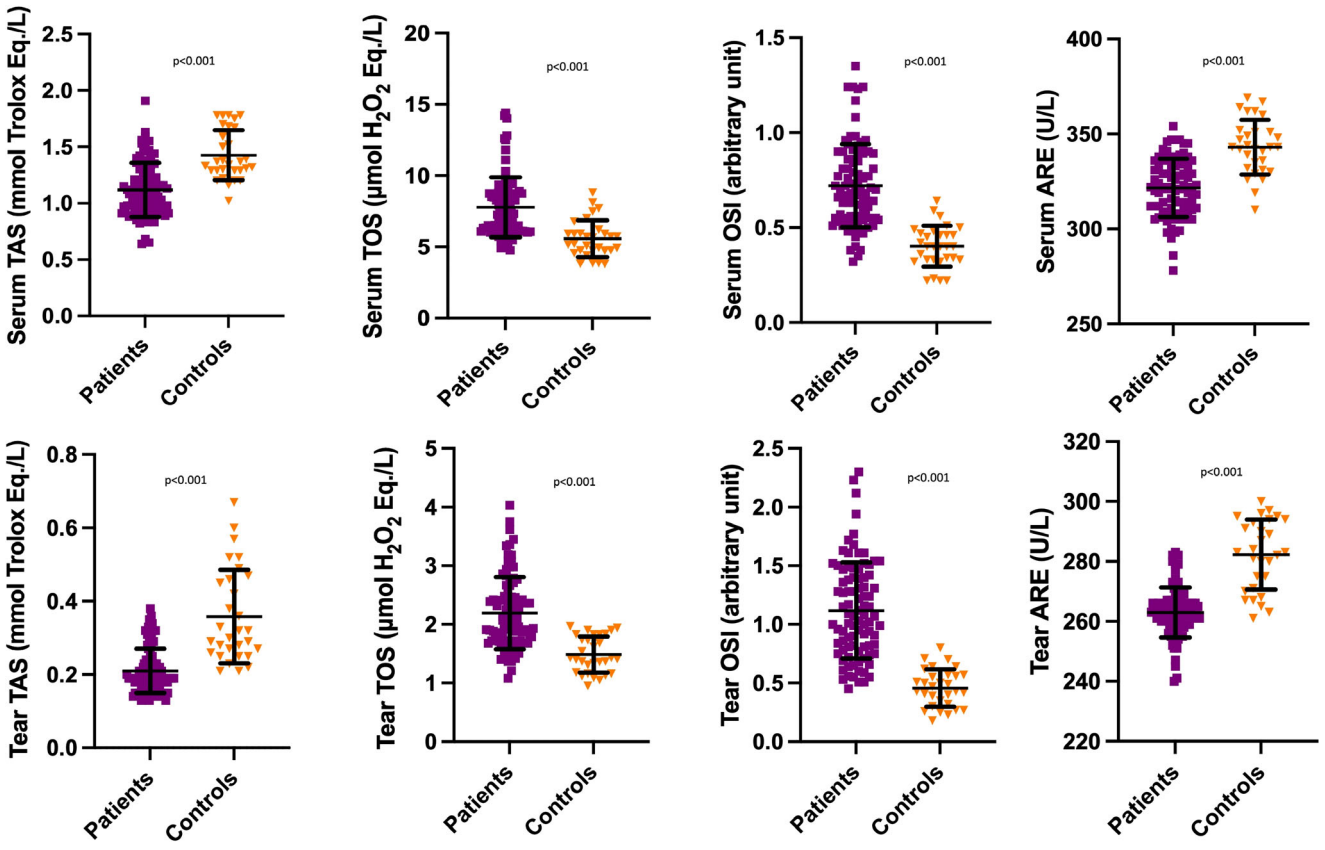

Figure 3. Comparison of serum and tear TAS, TOS, OSI and ARE levels between patients and controls. All serum and tear TAS, TOS, OSI and ARE levels were significantly different between patients and controls ($P < 0.001$).

Investigative Ophthalmology & Visual Science

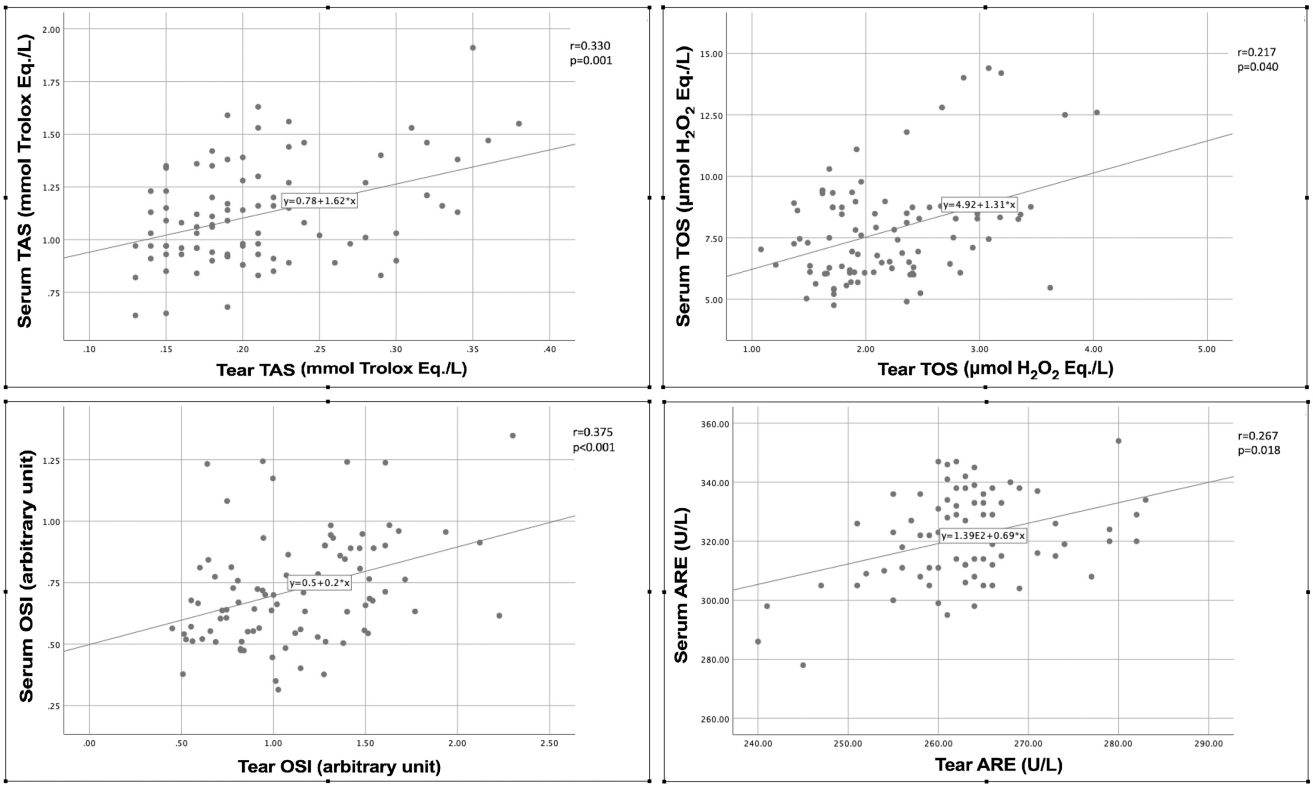

Figure 4. Correlation of TAS, TOS, OSI and ARE levels of patients between serum and tear samples. There were significant positive correlations between serum and tear TAS, TOS, OSI and ARE levels of patients ($P < 0.05$).

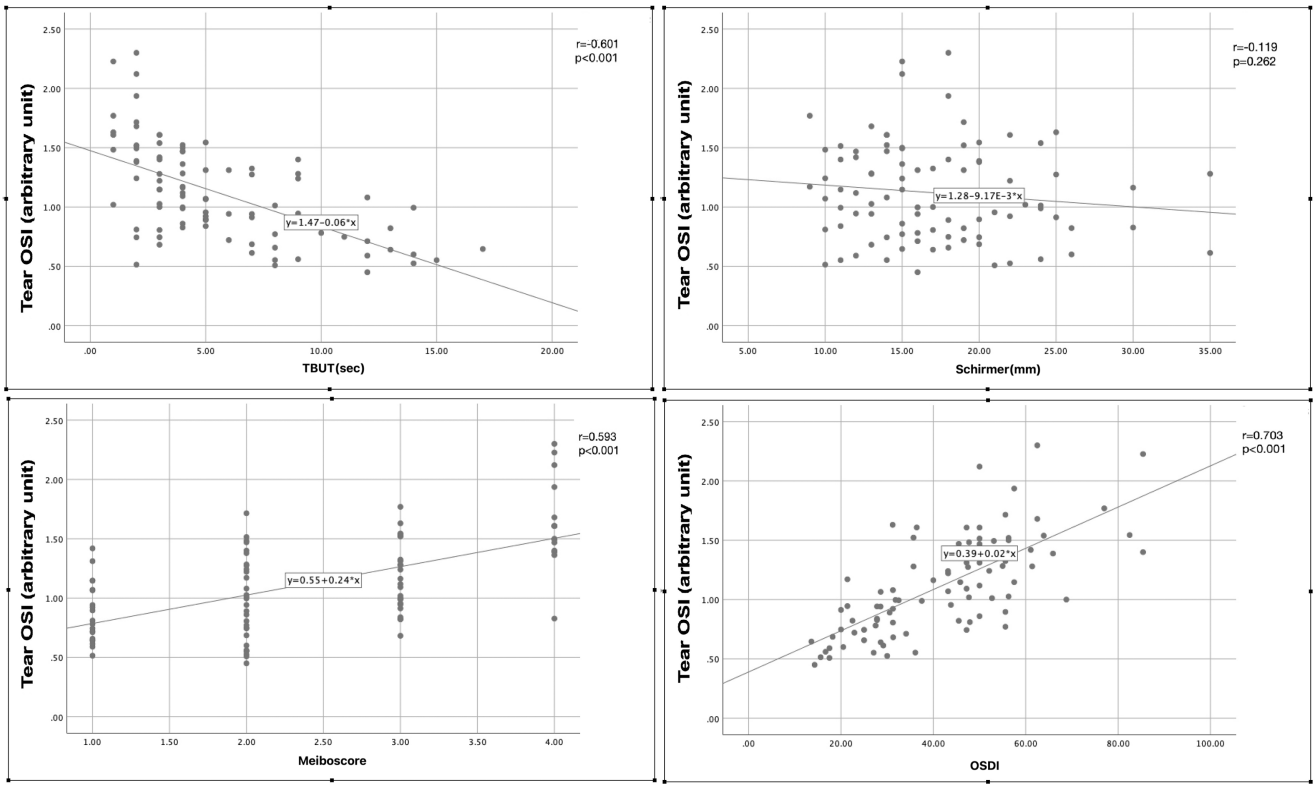

Figure 5. Correlation of tear OSI levels with ocular surface parameters. There were significant positive correlations between tear OSI and meiboscore, and between tear OSI and OSDI score, while a negative correlation between tear OSI and TBUT ($P < 0.01$). There was no correlation between tear OSI and Schirmer score ($P = 0.26$).

Investigative Ophthalmology & Visual Science

Table 3. Comparison of Oxidative Stress Parameters in Patients According to Skin Type

	Phymatous ($n = 15$)	Erythematotelangiectatic ($n = 46$)	Papulopustular ($n = 29$)
TAS serum	1.08 ± 0.28	1.12 ± 0.23	1.14 ± 0.24
TOS serum	8.39 ± 2.38	7.40 ± 1.96	8.06 ± 2.14
ARE serum	317.33 ±19.32	323.89 ± 15.41	320.89 ± 12.53
OSI serum	0.82 ± 0.28	0.69 ± 0.20	0.72 ± 0.20
TAS tear	0.19 ± 0.03	0.22 ± 0.06	0.21 ± 0.06
TOS tear	2.41 ± 0.74	2.15 ± 0.56	2.15 ± 0.62
ARE tear	261.64 ± 8.91	265.19 ± 8.70	261.90 ± 7.75
OSI tear	1.32 ± 0.44	1.05 ± 0.36	1.12 ± 0.45

No statistically significant difference between the groups.

samples in the patient group. There were positive statistical correlations ($P < 0.01$) between tear and serum values for each parameter. In addition, when we looked at the results in the control group, no correlation was found between tears and serum oxidative stress values ($P > 0.05$).

Table 1 shows the comparison of ocular surface parameters (TBUT, Schirmer, Meiboscore, and OSDI scores) between patients and controls. There were significant differences between patients and controls with respect to TBUT, Meiboscore, and OSDI scores ($P < 0.010$), although there was no difference with respect to Schirmer score between the groups ($P = 0.34$). Correlation analyzes investigating the relationship between oxidative stress level in tears (OSI) and ocular surface parameters are given in Figure 5. A negative correlation between tear OSI and TBUT was found whereas a positive correlation was established between OSI and Meiboscore and also between OSI and OSDI ($P < 0.01$). No correlation was found between the Schirmer test score and OSI ($P = 0.26$).

Last, when the ocular rosacea patients were subdivided into subgroups according to their skin types, we attributed 15 patients to the phymatous group, 46 patients to the erythematotelangiectatic group, and 29 patients to the papulopustular group. Table 3 shows TAS, TOS, OSI, and ARE values among ocular rosacea patients' skin-type subgroups. Even though TOS and OSI values tend to be higher in the phymatous group, no statistical difference was found between the two other groups and oxidative stress parameters.

Discussion

In this study, we evaluated TOS, TAS, OSI and ARE from tear and serum samples of ocular rosacea patients and compared those with the samples of healthy controls. We also investigated the association of this oxidative stress markers in tears with serum levels for each participant. According to our results, TOS activity and OSI level, which are reproducible markers of oxidative stress, were higher in ocular rosacea patients compared to the control group in both the tears and the serum. Conversely, TAS and ARE activities, which indicate antioxidant effects, were lower in ocular rosacea patients compared to controls in both the tears and the serum. The correlation analysis between the tear and serum levels showed some significant and positive correlations for TAS, TOS, OSI, and ARE values. With these results, we can argue that oxidative stress is associated with both skin rosacea and ocular rosacea.

Comparable to our study, studies investigating the role of oxidative stress in skin rosacea showed high levels of ROS and they generally associated this with ultraviolet radiation exposure.[9,10,24–26] It has also been suggested that skin inflammation in rosacea is triggered by elevated ROSs that cause oxidative modification of proteins and lipids.[25] Lipid peroxidation occurs when ROS react with polyunsaturated fatty acids in cell membranes, resulting in the formation of lipid peroxides and other reactive aldehydes.[27] Malondialdehyde is a commonly used lipid peroxidation marker that can be detected in tissues and body fluids. It has also been observed at high levels in cutaneous rosacea.[11] However, the reliability of malondialdehyde is still debated because it can be affected by various factors such as age, gender, smoking status, and dietary habits.[28]

On the other hand, recent studies have suggested that decreased levels of antioxidant enzymes may also play a role in the development of rosacea. For instance, serum superoxide dismutase, glutathione peroxidase, paraoxonase and arylesterase activities are lower in rosacea patients compared to healthy controls.[11,12,29] In our study, we specifically aimed to investigate ARE activity, because low ARE levels have been detected in retina, lens and aqueous humor in other eye diseases; however, it has never been reported in tears. During our preliminary studies, we observed that ARE activity can be measured even in small amounts of tears. The positive correlation between ARE activities in the tears and in the serum corroborates that ARE activity can be reliably measured in tears.

In recent years, measurement of TAS and TOS levels have been performed with a newly developed method, which is a spectrophotometric fully automatic calorimetric method and preferred in many studies to screen overall oxidative stress in tissues.[13,14] Guler et al.[15] found lower serum TAS levels and higher serum TOS levels in skin rosacea patients compared to healthy controls, which is consistent with our serum sample results. Using a similar design, Erdogan et al. observed higher serum TAS levels (possibly resulting from a compensatory mechanism in mild forms), as well as higher TOS levels in cutaneous rosacea patients.[16] In addition, TAS and TOS levels were investigated between cutaneous subtypes in the study, and no significant difference was found between the erythematotelangiectatic and papulopustular subtypes for these parameters.[16] In our study, we did not find significant difference between the phymatous, erythematotelangiectatic and papulopustular ocular rosacea subtypes, in serum as well as in tears' oxidative stress parameters (TAS, TOS, OSI, and ARE). These results suggest that oxidative stress does not result from a specific rosacea subtype but could rather play a role in the pathogenesis of all rosacea subtypes.

Investigative Ophthalmology & Visual Science

To better comprehend the clinical implications of oxidative stress in ocular rosacea, we assessed tear OSI along with ocular surface parameters like TBUT, Schirmer test score, meiboscore, and OSDI. Interestingly, TBUT, meiboscore, and OSDI scores worsened as the oxidative stress score increased, although there was no correlation with the Schirmer score. Oxidative stress status seems to correlate with signs of MGD but not with the aqueous phase of tear production.

Because the role of oxidative stress in skin rosacea has been shown in the literature before, it was not a surprise for us to see that it also plays a role in ocular rosacea. Besides, we found that the systemic and the tear oxidative stress levels were well correlated, enabling us to more clearly reveal the role of oxidative stress in the pathogenesis of the disease. In addition, as it is known and shown in this study, ocular rosacea is a serious cause of dry eye (especially because it causes MGD and therefore evaporative type of dry eye). In the literature, dry eye disease from other causes has also been shown to be associated with oxidative stress.[30,31] Considering that both rosacea is associated with oxidative stress and the resulting dry eye is associated with oxidative stress in our study, we can talk about the presence of a significant oxidative stress disorder in this disease. Perhaps we would have shown more clearly the potential extra effect of oxidative stress in ocular rosacea if we also included dry eye patients due to other causes without rosacea as a control group and compared them. Our work can be supported by showing this in future studies.

We did not explore rosacea patients with advanced ocular involvements such as keratitis, limiting our conclusion to uncomplicated forms of ocular rosacea. Most of the advanced cases with serious ocular complications first applied to the emergency department, and their treatment was started until they were evaluated in our corneal unit. Because of the challenges associated with catching untreated patients with advanced ocular involvement and the resulting difficulty in obtaining a statistically significant number of such patients, we opted not to include this particular group in the study. In addition, we did not include ocular rosacea patients without skin involvement, because mild-to-moderate ocular rosacea patients without skin rosacea diagnosed were coming with dry eye symptoms and were easily overlooked. Therefore it was easier for us to identify those with ocular rosacea among patients with skin rosacea, and thus we could be more confident in the diagnosis.

To conclude, oxidative stress appears to play concurrent and significant roles in both skin rosacea and ocular rosacea. We observed a strong correlation between tear and serum oxidative stress levels in ocular rosacea. Tear oxidative stress levels were also related to ocular surface parameters particularly those associated with MGD, a hallmark of ocular rosacea, although we did not observe any difference between the skin rosacea subtypes in terms of tear and serum oxidative stress levels. Future studies including a higher number of patients in each subgroup may confirm these results.

Acknowledgments

Supported by Gazi University Scientific Research Projects Unit.

Disclosure: **N. Yesilirmak**, None; **N. Bukan**, None; **B. Kurt**, None; **S. Yuzbasioglu**, None; **M. Zhao**, None; **D. Rodrigues-Braz**, None; **A. Aktas**, None; **F. Behar-Cohen**, None; **J.-L. Bourges**, None

References

1. Gold LM, Draelos ZD. New and emerging treatments for rosacea. *Am J Clin Dermatol*. 2015;16:457–461.
2. Redd TK, Seitzman GD. Ocular rosacea. *Curr Opin Ophthalmol*. 2020;31:503–507.
3. Tavassoli S, Wong N, Chan E. Ocular manifestations of rosacea: a clinical review. *Clin Exp Ophthalmol*. 2021;49:104–117.
4. Steinhoff M, Buddenkotte J, Aubert J, et al. Clinical, cellular, and molecular aspects in the pathophysiology of rosacea. *J Investig Dermatol Symp Proc*. 2011;15:2–11.
5. Woo YR, Lim JH, Cho DH, Park HJ. Rosacea: molecular mechanisms and management of chronic cutaneous inflammatory condition. *Int J Mol Sci*. 2016;17:1562.
6. Rainer BM, Kang S, Chien AL. Rosacea: epidemiology, pathogenesis, and treatment. *Dermatoendocrinol*. 2017;9(1):e1361574.
7. Pizzino G, Irrera N, Cucinotta M, et al. Oxidative stress: harms and benefits for human health. *Oxid Med Cell Longev*. 2017;2017:8416763.
8. Juan CA, Pérez de la Lastra JM, Plou FJ, Pérez-Lebeña E. The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *Int J Mol Sci*. 2021;22:4642.
9. Tisma VS, Basta-Juzbasic A, Jaganjac M, et al. Oxidative stress and ferritin expression in the skin of patients with rosacea. *J Am Acad Dermatol*. 2009;60:270–276.
10. Baz K, Cimen MY, Kokturk A, et al. Plasma reactive oxygen species activity and antioxidant potential levels in rosacea patients: correlation with seropositivity to Helicobacter pylori. *Int J Dermatol*. 2004;43:494–497.
11. Oztas MO, Balk M, Ogüs E, Bozkurt M, Ogüs IH, Ozer N. The role of free oxygen radicals in the aetiopathogenesis of rosacea. *Clin Exp Dermatol*. 2003;28:188–192.
12. Takci Z, Bilgili SG, Karadag AS, Kucukoglu ME, Selek S, Aslan M. Decreased serum paraoxonase and arylesterase activities in patients with rosacea. *J Eur Acad Dermatol Venereol*. 2015;29:367–370.
13. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem*. 2004;37:277–285.
14. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem*. 2005;38:1103–1111.
15. Guler EM, Ozkan BN, Yilmaz N, et al. Increased oxidative stress and imbalance dynamic thiol–disulfide homeostasis in Rosacea. *Turk J Dermatol*. 2022;16:120–124.
16. Erdogan HK, Bulur I, Kocaturk E, Saracoglu ZN, Alatas O, Bilgin M. Advanced oxidation protein products and serum total oxidant/antioxidant status levels in rosacea. *Postepy Dermatol Alergol*. 2018;35:304–308.
17. Tan J, Almeida LM, Bewley A, et al. Updating the diagnosis, classification and assessment of rosacea: recommendations from the global ROSacea COnsensus (ROSCO) panel. *Br J Dermatol*. 2017;176:431–438.
18. Craig JP, Nichols KK, Akpek EK, et al. TFOS DEWS II definition and classification report. *Ocul Surf*. 2017;15:276–283.
19. Bron AJ, de Paiva CS, Chauhan SK, et al. TFOS DEWS II pathophysiology report. *Ocul Surf*. 2017;15:438–510.
20. Muhafız E, Aslan Bayhan S, Bayhan HA, Çölgeçen E, Gürdal C. Evaluation of meibomian glands in cutaneous rosacea. *Med J SDU*. 2021;28:621–626.

21. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the ocular surface disease index. *Arch Ophthalmol*. 2000;118:615–621.
22. Balmus IM, Alexa AI, Ciuntu RE, et al. Oxidative stress markers dynamics in keratoconus patients' tears before and after corneal collagen crosslinking procedure. *Exp Eye Res*. 2020;190:107897.
23. Haagen L, Brock A. A new automated method for phenotyping arylesterase (EC 3.1.1.2) based upon inhibition of enzymatic hydrolysis of 4-nitrophenyl acetate by phenyl acetate. *Eur J Clin Chem Clin Biochem*. 1992;30:391–395.
24. Jones D. Reactive oxygen species and rosacea. *Cutis*. 2004;74(3 suppl):17–20, 32–34.
25. Jones DA. Rosacea, reactive oxygen species, and azelaic acid. *J Clin Aesthet Dermatol*. 2009;2:26–30.
26. Sundaram C, Köster W, Schallreuter KU. The effect of UV radiation and sun blockers on free radical defence in human and guinea pig epidermis. *Arch Dermatol Res*. 1990;282:526–531.
27. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev*. 2014;2014:360438.
28. Khoubnasabjafari M, Ansarin K, Jouyban A. Reliability of malondialdehyde as a biomarker of oxidative stress in psychological disorders. *Bioimpacts*. 2015;5:123–127.
29. Yazici AC, Tamer L, Ikizoglu G, Kaya TI, Api H, Yildirim H. GSTM1 and GSTT1 null genotypes as possible heritable factors of rosacea. *Photodermatol Photoimmunol Photomed*. 2006;22:208–210.
30. Dogru M, Kojima T, Simsek C, Tsubota K. Potential role of oxidative stress in ocular surface inflammation and dry eye disease. *Invest Ophthalmol Vis Sci*. 2018;59(14):DES163–DES168.
31. Seen S, Tong L. Dry eye disease and oxidative stress. *Acta Ophthalmol*. 2018;96(4):e412–e420.