

# Dynamic Thiol/Disulfide Balance in Patients with Seborrheic Dermatitis: A Case–Control Study

Selma Emre, Göknur Kalkan, Serpil Erdoğan<sup>1</sup>, Akın Aktaş, Merve Ergin<sup>1</sup>

Departments of Dermatology and <sup>1</sup>Biochemistry, Faculty of Medicine, Ankara Yıldırım Beyazıt University, Atatürk Training and Research Hospital, Ankara, Turkey

## Abstract

**Background:** Seborrheic dermatitis is a chronic, inflammatory skin disease, in which many endogenous and exogenous factors play a role. Recent studies have shown that oxidative stress increases in these patients. The role of the dynamic thiol/disulfide homeostasis, an important component of the oxidative stress, in the pathogenesis of seborrheic dermatitis has not yet been investigated.

**Objectives:** The objective was to investigate the relationship between the dynamic thiol/disulfide balance in the plasma of seborrheic dermatitis patients and disease severity.

**Methods:** In this case–control study, 70 seborrheic dermatitis patients and 61 age- and sex-matched healthy controls were included. Thiol/disulfide homeostasis was calculated from venous blood samples, and tests were performed by automated spectrophotometric method. The thiol/disulfide balance between the patient and control groups was compared. In addition, disease severity and other demographic characteristics and thiol/disulfide balance parameters were compared.

**Results:** Native and total thiols were significantly higher in the patient group than that in the control group ( $P < 0.001$ ). Disulfide levels were nonsignificantly lower in the patient group than controls ( $P = 0.821$ ). Patients' age and age at the onset of disease were found to have a negative correlation with native and total thiol levels.

**Conclusion:** Higher levels of thiols in the serum may be responsible for the increased proliferation of seborrheic dermatitis lesions. To the best of the authors' knowledge, this is the first report on the correlation between thiol/disulfide homeostasis in patients with seborrheic dermatitis.

**Keywords:** Disease severity, hyperproliferation, oxidative stress, seborrheic dermatitis, thiol/disulfide

**Address for correspondence:** Prof. Selma Emre, Ankara Yıldırım Beyazıt Üniversitesi, Tıp Fakültesi, Dermatoloji Anabilim Dalı, Eskişehir yolu 7 km, 06800 Çankaya, Ankara, Turkey.

E-mail: dr\_semre@yahoo.com

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## INTRODUCTION

Seborrheic dermatitis (SD) is a common chronic recurrent inflammatory skin disease. It is characterized by greasy scale, pink-red, itchy lesions in the body's seborrheic parts such as scalp, nasolabial folds, eyebrows, chin, ears and interscapular

and presternal areas.<sup>[1,2]</sup> This disease has a wide-ranging severity and changing course, depending on several triggering factors such as age, gender, obesity, seasonal changes, alcohol, smoking, psychological stress, depression, HIV and neurological diseases such as Parkinson's disease.<sup>[3]</sup>

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The etiopathogenesis of SD is not fully understood. It is considered to be a multifactorial disease where several endogenous and exogenous factors play a role. *Malassezia* yeast is one of the most important predisposing factors in SD. Toxic metabolites released by *Malassezia* yeasts activate the host's immune response by irritating the skin, which leads to deterioration of epidermal differentiation.<sup>[4,5]</sup> However, whether its presence is incidental or causative is not known. In addition, it has been suggested that inflammatory responses in SD are initiated by personal tendency, nonimmunogenic irritations and oxidative stress. Oxidative stress can occur because of either overproduction of oxidant molecular reactive oxygen species (ROS) and hydroxyl radicals (OH<sup>-</sup>) or insufficiency of antioxidant mechanisms.<sup>[6]</sup>

Thiols are the most important molecules in eradicating ROS by enzymatic or nonenzymatic mechanisms. Thiol groups create disulfide bonds due to the existence of ROS in the environment. Conversion of thiols to disulfide is the earliest indication of ROS-mediated protein oxidation. Thiol groups converted to reversible disulfide structures can again be reduced to thiol groups, and thus the thiol/disulfide balance can be maintained. Thiol/disulfide balance is dynamic, and the most important parameters of this homeostasis condition are total thiols, native thiols and disulfide. When this balance deteriorates, it can lead to several inflammatory diseases.<sup>[6,7]</sup> Few recent studies have shown that the balance of oxidative stress is impaired in patients with SD, and thus there may be a relationship between oxidative stress and inflammatory responses in this disease.<sup>[8,9]</sup>

However, current studies are not sufficient to understand oxidant and antioxidant balance. As dynamic thiol/disulfide balance is one of the most important components of oxidative stress in patients with SD, this study was conducted to investigate the relationship between the plasma thiol levels in SD patients and disease severity.

## METHODS

This case-control study included SD patients aged 18–65 years who attended the Dermatology Clinic of Ankara Atatürk Training and Research Hospital between March 2015 and March 2016 and met the inclusion criteria. SD was diagnosed by clinical and/or histopathological examination.

Patients who were pregnant or lactating; HIV positive; had a neurologic, malignant, active liver, kidney or infectious disease; or were taking drugs for other diseases were not included in this study, as their condition could alter the

studied parameters and impact the validity of the results. A total of 70 patients met the inclusion criteria during the study period and agreed for participation in the study. Subsequently, 61 age- and sex-matched healthy controls were recruited from the hospital staff or relatives of the patients.

This study was approved by the hospital's local ethics committee (No. 26379996/19) on January 14, 2015, and was carried out in accordance with the guidelines of the Declaration of Helsinki, 2013. The patients and healthy volunteers were informed about the study and provided a signed informed consent prior to commencement of the study.

Duration of disease, smoking habits and education levels of the patients were noted. Disease severity was determined from the Seborrheic Dermatitis Area Severity Index.<sup>[10]</sup>

Thiol/disulfide homeostasis parameters were calculated from venous blood samples. The venous blood samples were taken early morning after at least an 8-h overnight fasting. Then, the samples were centrifuged at 3600 rpm for 10 min and sera were stored at  $-80^{\circ}\text{C}$  (Sanyo, Tokyo, Japan) until all samples were collected and assayed at the same time.

## Thiol/disulfide homeostasis parameter measurement

Serum native and total thiol concentrations indicating thiol/disulfide homeostasis were calculated using a novel automated colorimetric method developed by Erel and Neselioglu.<sup>[11]</sup> To measure the level of total thiol, dynamic disulfide bonds were firstly reduced to functional thiol groups by adding a reducing agent sodium borohydride ( $\text{NaBH}_4$ ). Formaldehyde was added, and unused reductant  $\text{NaBH}_4$  was exhausted. All thiol groups, containing reducing and native thiols, were determined after reaction with 5,5'-dithiobis-(2-nitrobenzoic acid). The dynamic disulfide amount was determined as half the difference between the total thiols and native thiols (i.e., total thiol – native thiol/2). All evaluations were performed by a single evaluator, and the evaluator was blinded to the sample group.

After the determination of native and total thiols, the disulfide amounts, disulfide/total thiol percent ratios ( $\text{SS}/\text{SH} + \text{SS}$ ), disulfide/native thiol percent ratios ( $\text{SS}/\text{SH}$ ) and native thiol/total thiol percent ratios ( $\text{SH}/\text{SH} + \text{SS}$ ) were calculated.

## Statistical analysis

The PASW Statistics 18 software (IBM Corporation, Armonk, New York, USA) was used for statistical analysis

of data. Kolmogorov–Smirnov test was used to examine the distribution pattern of the variables. Variables exhibiting a normal distribution were compared by the Student's *t*-test, and values were expressed as mean  $\pm$  standard deviation. The parameters having an abnormal distribution were compared with the Mann–Whitney U-test, and values were expressed as medians (interquartile range). Categorical variables were expressed as percentages. Pearson's Chi-square tests were used to compare categorical variables.  $P < 0.05$  was considered statistically significant.

A correlation between test results and demographic data of patients' analyses was evaluated with the Spearman's test due to abnormality of distribution of these parameters.  $P < 0.05$  was considered indicative of statistical significance.

## RESULTS

A total of 70 patients were included in the study, of which 47 were male and 23 were female. Among the 61 healthy controls, 34 were male and 27 were female. Patient and control groups were not different in terms of age and gender. The age of patients ranged from 18 to 63 (mean:  $31.87 \pm 11.13$ ) years. The demographic- and age-wise distribution of the patients is summarized in Table 1. The age of volunteers in the control group ranged from 19 to 67 (mean:  $35.82 \pm 13.04$ ) years.

**Table 1: Demographic characteristics of the patients**

Characteristics	n (%)
Gender	
Female	23 (32.9)
Male	47 (67.1)
Age (years)	
18–25	22 (31.4)
26–40	31 (44.3)
41–65	17 (24.3)
Age of onset	
<18	10 (14.3)
18–25	27 (38.6)
26–40	25 (35.7)
41–65	8 (11.4)
Education	
No literacy	1 (1.4)
Primary school	13 (18.6)
High school	26 (37.1)
University	26 (37.1)
Master's degree	4 (5.7)
Smoking	
Yes	20 (28.6)
No	50 (71.4)
Cholesterol*	
Normal	51 (76.1)
High	16 (23.9)
Triglyceride*	
Normal	53 (79.1)
High	14 (20.9)

\*Lipid profiles were not conducted in three patients

Native thiol and total thiols were found to be significantly higher in the patient group than that in the control group (both  $P < 0.001$ ). Disulfide levels were found to be insignificantly lower in the patient group than that in the control group ( $P = 0.821$ ). Comparison of thiol/disulfide homeostasis in patient and control groups is shown in Table 2.

In terms of correlation between thiol/disulfide balance and patient characteristics, the age of SD patients was found to be negatively correlated with native thiol and total thiol levels. Similarly, age at the onset of disease was found to be negatively correlated with native thiol and total thiol levels. In contrast, duration and severity of the disease were found to have a significant relationship with thiol/disulfide levels [Table 3].

## DISCUSSION

SD is one of the most common dermatological diseases, but its etiopathogenesis is yet to be fully understood. It is well known that there are endogenous and exogenous triggering factors; however, the mechanism of how these factors initiate an inflammatory reaction is not clearly known. In previous studies, it was reported that there is an increase in oxidative stress in the serum of patients with active SD.<sup>[8,12]</sup> In studies conducted on patients with psoriasis and atopic dermatitis, inflammatory skin diseases with similar characteristics to SD, the total and native thiols in the patient group were higher than that in the healthy controls.<sup>[6,13]</sup> In children with atopic dermatitis, thiols were found to be nonsignificantly higher in the patient group than in healthy children, whereas disulfide levels were significantly higher in the patient group than in healthy children.<sup>[13]</sup>

Recently, the hyperproliferative theory has gained importance in the pathogenesis of SD. This view was influenced by the fact that squam in SD did not respond to amphotericin B and keratolytic treatments and corticosteroids.<sup>[1]</sup> The pathophysiological characteristics of SD are very similar to those of psoriasis. Parakeratosis, increased keratinocyte turnover rate, hyperplasia and deteriorated barrier functions are the similar characteristics of SD and psoriasis. Tumor necrosis factor- $\alpha$ , interleukin (IL)-1  $\alpha$ , IL-1-R $\alpha$ , IL-8 and interferon- $\gamma$  from inflammatory cytokines with T helper cells that play a role in inflammation are the molecules participating in the pathophysiology of SD.<sup>[14]</sup> In a previous study examining the thiol/disulfide levels in patients with psoriasis, it was reported that thiol levels in severe psoriasis were higher than that in patients with mild psoriasis, but this difference was not statistically significant.<sup>[15]</sup> In another

**Table 2: Comparison of the patients and control groups**

Characteristics	Patient group (n = 70)	Control group (n = 61)	P
Gender, n (%)			
Male	47 (67.1)	34 (55.7)	0.180
Female	23 (32.9)	27 (44.3)	
Age (years), median, (IQR)	29.5 (17)	32 (22)	0.083
Native thiol, mean ± SD	504.17 ± 50.97	468.37 ± 52.01	<0.001
Total thiol, mean ± SD	543.74 ± 53.43	508.62 ± 54.50	<0.001
Disulfide, mean ± SD	19.75 ± 9.14	20.09 ± 7.82	0.821
Disulfide/native, mean ± SD	3.96 ± 1.85	4.34 ± 1.77	0.231
Disulfide/total, mean ± SD	3.62 ± 1.59	3.95 ± 1.51	0.227
Native/total, mean ± SD	92.76 ± 3.18	92.10 ± 3.07	0.230

Bold indicates significant values. IQR – Interquartile range; SD – Standard deviation

**Table 3: Correlation of the thiol/disulfide homeostasis and basic features of the patients**

Characteristics	r, P					
	Native thiol	Total thiol	Disulfide	Disulfide/native thiol	Disulfide/total thiol	Native thiol/total thiol
Age	-0.440, <0.001	-0.464, <0.001	-0.094, 0.438	-0.019, 0.877	-0.019, 0.877	0.023, 0.852
Duration	0.012, 0.920	0.008, 0.945	-0.044, 0.720	-0.045, 0.711	-0.045, 0.711	0.052, 0.669
Age of onset	-0.421, <0.001	-0.452, <0.001	-0.094, 0.441	-0.019, 0.876	-0.019, 0.876	0.021, 0.860
SDASI	-0.061, 0.618	-0.119, 0.326	-0.130, 0.285	-0.128, 0.291	-0.128, 0.291	0.129, 0.286

Bold indicates significant values. SDASI – Seborrheic Dermatitis Area Severity Index

study, native and total thiol levels in patients with psoriasis were found to be significantly higher than that in healthy individuals.<sup>[6]</sup> The results of the current study in patients with SD were similar to those with psoriasis patients, as the native and total thiols were also found to be significantly higher in the SD patient group than the control group.

In another study, oxidative stress parameters in scratch samples taken from the scalp of patients with SD were investigated, and superoxide dismutase, catalase and malondialdehyde levels were found to be significantly higher in these patients compared with healthy individuals. The investigators suggested that these antioxidant molecules can be increased as a defense mechanism against the superoxide radicals increased in the scalp of SD patients.<sup>[9]</sup> Similarly, our study found that thiols, which are also antioxidant products, were significantly higher in the patient group than that in the healthy controls.

Erel and Neselioglu<sup>[11]</sup> reported that the plasma thiol/disulfide balance shifts toward thiol in proliferative diseases such as multiple myeloma, bladder cancer, colon cancer and kidney cancer. In a very recent study, it was reported that in basal cell carcinoma, which is a proliferative disease, native thiol was found to be significantly higher in patients than that in the control group, whereas disulfide levels were lower in the patient group.<sup>[16]</sup> The increase in antioxidant products could have been as a defense mechanism against the oxidative stress in patients. Furthermore, it is known that thiol groups in

higher amounts play a role in hyperproliferative diseases. Glutathione (GSH) is the most important source of thiol groups in humans and is the most important endogenous antioxidant that can be found everywhere in the body.<sup>[17,18]</sup> It is known that there is a relationship between GSH concentrations in cells and cell proliferation, with cell proliferation increasing with increase in the levels of GSH and GSH precursors in cells.<sup>[19]</sup> Increased proliferation in patients with psoriasis and SD can be responsible for the higher thiol levels identified in these patients. To understand this hypothesis better, investigation and comparison of thiol/disulfide balance in active and remission periods of the disease can be helpful.

In the current study, the age at presentation and at the onset of disease were found to be negatively correlated with the native thiol and total thiol levels. As previous studies did not carry out such correlational analysis, comparisons cannot be made. The authors recommend future studies to include these parameters in analysis for a better understanding regarding such correlations. The authors also recommend further studies individually investigating and comparing other oxidative stress parameters outside thiol/disulfide to enlighten the subject.

A limitation of this study is that thiol/disulfide balance could not be investigated during both the active and remission periods of patients with SD. In addition, new aspects such as the correlation of thiols with causative agents of *Malassezia* group could not be investigated.

## CONCLUSION

To the best of the authors' knowledge, this is the first report on the correlation between thiol/disulfide homeostasis in patients with SD. This study found that the level of thiol groups is higher in the serum of patients with active SD than those in healthy individuals. Increase in thiol levels in serum may be due to an effect of homeostasis to reduce the increased burden of oxidative stress in SD patients, and increased thiol groups may be responsible for increased proliferation of SD lesions.

## Ethical considerations

This study was approved by the Ethics Committee of Ankara Atatürk Training and Research Hospital (No. 26379996/19) on January 14, 2015. The study was conducted in adherence with the guidelines of the Declaration of Helsinki, 2013. Signed informed consent was obtained from all patients and volunteers before inclusion in this study.

## Peer review

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## Conflicts of interest

There are no conflicts of interest.

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