

A new oxidative stress marker for thiol-disulphide homeostasis in seasonal allergic rhinitis

Seckin Ulusoy, M.D.,¹ Nilhan Nurlu Ayan, M.D.,² Mehmet Emre Dinc, M.D.,¹ Abdulhalim Is, M.D.,¹ Cemile Bicer, M.D.,³ and Ozcan Erel, M.D.³

ABSTRACT

Background: Reactive oxygen species has a crucial role in the pathogenesis of many diseases. Reactive oxygen species has been effectively documented in patients with asthma, but very little research has been reported in subjects with allergic rhinitis.

Objectives: To investigate thiol (SH)–disulfide (SS) homeostasis, a new oxidative stress marker present in patients with seasonal allergic rhinitis (SAR).

Methods: A total of 64 subjects, including 32 patients with SAR and 32 healthy controls, were included in the study. We measured the SH levels as a marker of antioxidant protection and SS as a marker related to oxidant stress. Sera specimens were taken from patients with SAR during exacerbation and during asymptomatic time periods. In addition, sera samples from the control group were also obtained during the pollen season to compare with those from patients with SAR.

Results: In patients with SAR, during exacerbation periods, SH, SS, % SS/SH, % SS to total SH (TT), and % SH/TT were significantly different ($p < 0.05$) than in patients with SAR during asymptomatic periods. Although SS was significantly higher in patients with SAR during exacerbation periods, SH was significantly higher in patients with SAR during asymptomatic periods. Patients with SAR during asymptomatic periods and the control group did not significantly differ ($p > 0.05$) regarding SH, SS, TT, % SS/SH, % SS/TT, and % SH/TT.

Conclusion: SAR is a disorder that elevates systemic oxidative stress and reduces antioxidant enzyme activities. Our results shed light on the etiopathogenesis of the disease and can help develop new therapeutic approaches.

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Allergic rhinitis (AR) is a very common health disorder that causes problems for 10–40% of the human population worldwide, and its frequency is growing both in adults and children.¹ The respiratory system consists of superior and lower portions, which together form a special anatomic-functional component to produce the well-known “one airway one disease” model.² Recent effective studies reported that AR and asthma coexist within a notable percentage of patients with atopy and stated that they reveal familiar pathogenetic pathways and inflammation properties.^{3–5}

Oxidative stress (OS) is the expression of a disproportionate amount of reactive oxygen species (ROS) and the deoxidizing systems of the affected cells that eliminate these types of radicals.^{5–7} During physiologic situations, the production of ROS is not damaging to cells and it is restricted by antioxidants. However, when the degree of ROS exceeds pathologic amounts, the antioxidants cannot reduce the harmful effects.⁸ Thiols (SH), also more commonly known as mercaptans, consist of sulfhydryl groups.⁹ The blood SH pool is primarily produced by albumin SHs and protein SHs, and is partly established by low-molecular-weight SHs that include cysteine, cysteinylglycine, glutathione, homocysteine, and γ glutamylcysteine.^{9,10}

SH can proceed through an oxidation reaction *via* oxidants and develop disulfide (SS) chains. The organized SS connections can again be reduced to SH; consequently, dynamic SS-SH homeostasis is managed.^{9,11} As an accessory to the main systemic OS, there is a locally elevated oxidative load throughout the breathing passages.⁵ Inflammatory tissues in the air passage that include macrophages and eosinophils were demonstrated to generate increased quantities of ROS.^{5,12} Most of these ROS, which include superoxide radicals, hy-

droxyl radicals, and hydrogen peroxide, aggravate inflammatory reactions, vascular permeability, and epithelial injury as well as respiratory tract hyperresponsiveness.⁶

Dynamic SH-SS homeostasis plays an important role in the removal of toxins and in antioxidant defense, transmission transduction, regulation of enzymic actions, apoptosis, and cell signaling pathways.^{9,13} OS has been effectively documented in patients with asthma^{6,7,12,14,15}; however, until now, very little research has been reported in subjects with AR.^{16,17} We established our hypothesis based on AR and asthma having similar pathologies and designed this prospective case-control study to identify oxidant and antioxidant conditions in SAR.

METHODS

Study Population

A total of 32 patients with SAR (12 women and 20 men; mean (standard deviation [SD]) age, 33.96 $\pm$ 9.5 years) presented to the otolaryngology outpatient clinic of the Gaziosmanpasa Taksim Training and Research Hospital, a tertiary reference hospital in Istanbul. Moreover, 32 age- and sex-matched (13 women and 19 men; mean (SD) age, 34.1 $\pm$ 9.6 years) healthy subjects without atopy were enrolled as the control group for comparison in our investigation (Table 1). The control group was seen in the outpatient department of the same hospital during the study period; healthy volunteers constituted the control group subjects. Initial blood tests were performed in our SAR group during the pollen season, from April to August, 2014 (SAR exacerbation period). After the pollen season, sera samples were prepared from a second blood test in November 2014 (SAR asymptomatic period).

Patients with SAR were enrolled during the pollen season and were involved in the study when they were symptomatic and before receiving any medications (none of these patients had received oral, inhaled, or nasal corticosteroids; antibiotics; topical or systemic antihistamines; or leukotriene antagonists in the 30 days before enrollment in the study). We excluded those patients with clinical proof of repeated or active air passage disease, concomitant asthma, and considerable comorbidities. We compared the patients with SAR during the exacerbation and asymptomatic periods (Table 2) with the control

From the ¹Department of Otorhinolaryngology, Gaziosmanpasa Taksim Education and Research Hospital, Istanbul, Turkey, ²Department of Clinical Biochemistry, Gaziosmanpasa Taksim Education and Research Hospital, Istanbul, Turkey, and ³Department of Clinical Biochemistry Yildirim Beyazit University Faculty of Medicine, Ankara, Turkey

The authors have no conflicts of interest to declare pertaining to this article. Address correspondence to Seckin Ulusoy, M.D., Avrupa Trans Europe Motorway konutlari 12/72 Gaziosmanpasa, Istanbul, Turkey

E-mail address: seckinkbb@gmail.com

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group (Table 3) in terms of the following parameters: native SH, SS, total SH (TT), % SS/SH, % SS/TT, and % SH/TT.

Ethical Approval

The current study was carried out in accordance with the principles of the Declaration of Helsinki as well as good clinical training suggestions. Written, well-informed consent was received from each patient with SAR and control subject, and was approved by the local ethics committee at the Gaziosmanpasa Taksim Education and Research Hospital (August 20, 2014; protocol 53).

Diagnosis

SAR was identified based on the Allergic Rhinitis and its Impact on Asthma guidance by the existence of more than one of the signs and symptoms of AR (sneezing, watery rhinorrhea, nose blockage, nasal

pruritus) sustainable in excess of 1 hour on most days with a concordance between a characteristic history of allergic conditions as well as skin-prick diagnostic tests.^{1,18}

Skin-Prick Testing

Testing was carried out by using a panel composed of eight aeroallergens on the volar surface of the forearm, and the reaction was assessed and registered. The allergens used for the prick test (Prick test kit; Stallergenes S.A., Antony, France) comprised the following eight substances: (1) positive control, (2) negative control, (3) *Dermatophagoides pteronyssinus*, (4) *Dermatophagoides farinea*, (5) grass pollens, (6) a tree pollen combination, (7) cereal products, and (8) molds. Patients did not use antihistamines within the 10 days before undergoing the test. To achieve regular drilling, a prick lancet (Mizollen; H. Herenz GmbH, Hamburg, Germany) was adopted. A glycerol-buffer diluent was used as the negative control, and 10 mg/mL of histamine hydrochloride was applied as the positive control. The response was reviewed 20 minutes after application: an induration of >3 mm than that of the negative control was regarded as positive, and individuals with a minimum of 1 positive result were regarded as having SAR. We included in this study only (5) grass pollens, (6) a tree pollen combination and excluded other positive allergies.

Blood Sampling

The venous blood samples from the SAR and control groups were collected in the early morning after a minimum of 8 hours of overnight fasting. Subsequently, the trial samples were centrifuged at 3600 rpm for 6 minutes, and sera were saved at -80°C until just before usage for analysis. The SH measurement process was carried out by using an automated analyzer. Plasma SH, SS, and TT were determined by using a new, automated procedure described by Erel and Neselioglu⁹ In the initial step of the analysis, dynamic SS bonds in the

Table 1 Demographic characteristics of patients with SAR (case group) and healthy controls (control group)

	Case Group	Control Group	p
Age, y			0.957*
Mean ± SD	33.9 ± 9.5	34.1 ± 9.6	
Median (minimum-maximum)	35 (18–50)	35 (19–49)	
Sex, no. (%)			0.798**
Women	12 (37.5)	13 (40.6)	
Men	20 (62.5)	19 (59.4)	

SAR = seasonal allergic rhinitis; SD = standard deviation.

*Independent sample t test.

**χ² test.

Table 2 Comparison of SAR during the exacerbation and asymptomatic period based on SH, SS, TT, % SS/SH, % SS/TT, and % SH/TT

	SAR Exacerbation Period		SAR Asymptomatic Period		p*
	Mean ± SD	Med (min-max)	Mean ± SD	Median (min-max)	
Native SH	347.7 ± 49.2	357 (173–427)	355.5 ± 53.5	366 (238–441)	0.018
TT	394.5 ± 53.5	401 (210–468)	394.2 ± 59.1	405 (273–480)	0.212
SS	23.4 ± 5.2	23 (14–38)	19.4 ± 5.8	19 (7–29)	0.014
% SS/SH	6.8 ± 1.6	6 (5–11)	5.5 ± 1.5	6 (2–8)	0.001
% SS/TT	6.0 ± 1.2	6 (4–9)	4.9 ± 1.2	5 (2–7)	0.001
% SH/TT	88.1 ± 2.4	89 (82–92)	90.2 ± 2.4	90 (86–96)	0.001

SAR = seasonal allergic rhinitis; SH = thiol; SS = disulfide; TT = total SH; % SS/SH = disulphide/thiol percentage ratio; % SS/TT = disulphide/total thiol percentage ratio; % SH/TT = thiol/total thiol percentage ratio; SD = standard deviation; min = minimum; max = maximum.

*Wilcoxon test.

Table 3 Comparison of the control and case group based on SH, SS, TT, % SS/SH, % SS/TT and % SH/TT

	Control Group		Case Group					
			SAR Exacerbation Period			SAR Asymptomatic Period		
	Mean ± SD	Median	Mean ± SD	Median	p	Mean ± SD	Median	p*
Native SH	378.4 ± 39.6	384	347.7 ± 49.2	357	0.009	355.5 ± 53.5	366	0.114
TT	423.2 ± 41.8	428	394.5 ± 53.5	401	0.034	394.2 ± 59.1	405	0.097
SS	22.4 ± 6.8	21	23.4 ± 5.2	23	0.210	19.4 ± 5.8	19	0.184
% SS/SH	6.0 ± 2.0	6	6.8 ± 1.6	6	0.005	5.5 ± 1.5	5.5	0.844
% SS/TT	5.3 ± 1.5	5	6.0 ± 1.2	6	0.005	4.9 ± 1.2	5.0	0.844
% SH/TT	89.4 ± 3.0	90	88.1 ± 2.4	89	0.005	90.2 ± 2.4	90	0.844

SH = thiol; SS = disulfide; TT = total SH; % SS/SH = disulphide/thiol percentage ratio; % SS/TT = disulphide/total thiol percentage ratio; % SH/TT = thiol/total thiol percentage ratio; SD = standard deviation.

Mann-Whitney U test.

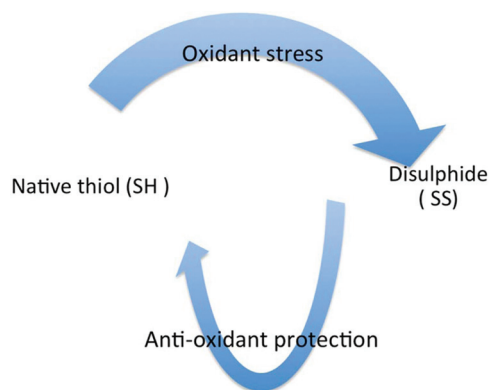


Figure 1. Disulphides (SS), native thiol (SH) circulation but total thiol (TT) remains unchanged.

serum sample were reduced to the free SH functional group with sodium borohydride. In the second step, the remaining unused reductant sodium borohydride was entirely absorbed. In the final step, all SH groups and reduced SH groups were measured after reaction with 5,5'-dithiobis-2-nitrobenzoic acid. In this study, SH-SS homeostasis was calculated by determining native SH and reducible dynamic SS amounts, whereas other relevant factors were determined by calculations.

The Statistical Package for Social Sciences for Windows version 22 (IBM SPSS Inc, Chicago, IL) was used for statistical evaluation of the data. The Kolmogorov-Smirnov test was used to analyze the distribution of the variables. Normally distributed mathematical variables were presented as means (SDs), whereas those not normally distributed were presented as medians (minimum-maximum). For detailed statistics of the data, the mean, SD, median, minimum, maximum, frequency, and rate amounts were used. The Wilcoxon test was used to evaluate repetitive calculations. To assess quantitative data, the Mann-Whitney *U* test and independent sample *t*-test was applied. The χ^2 test were used to evaluate qualitative data.

RESULTS

The sex and mean age of the subjects with SAR and the control group were not significantly different ($p > 0.05$) (Table 1). TT did not significantly differ ($p > 0.05$) in the SAR group during the exacerbation period versus the SAR group during the asymptomatic period. In the SAR group, during the exacerbation period, SH, SS, %SS/SH, %SS/TT, and %SH/TT differed significantly ($p < 0.05$) compared with the SAR group during the asymptomatic period. Although SS was significantly higher in the SAR group during the exacerbation period, SH was higher in the SAR group during the asymptomatic period (Table 2). The SAR group during the asymptomatic period and the control group were not significantly different ($p > 0.05$) with regard to SH, SS, TT, %SS/SH, %SS/TT, and %SH/TT values. In the SAR group during the exacerbation period, SH, TT, %SS/SH, %SS/TT, and %SH/TT values were significantly different ($p < 0.05$) compared with the control group. SH and TT levels were significantly lower ($p < 0.05$) in the SAR group during the exacerbation period compared with the control group. SS did not differ significantly ($p > 0.05$) in the SAR group during the exacerbation period compared with the control group (Table 3).

DISCUSSION

The principal findings of our study are as follows: first, in the SAR group during the exacerbation period, the patients exhibited decreased levels of SH and increased SS compared with the asymptomatic period. Second, for patients with SAR during the exacerbation period, SH and TT levels were lower than in the control group but the

SS level remained unchanged. Third, SH levels in the patients with SAR during the asymptomatic period were higher than during the exacerbation period but still lower than in the control group.

Our study showed that SH level was low in the SAR group during the asymptomatic period but not statistically so compared with the control group (355.5 $\mu\text{mol/L}$ in the SAR group during the asymptomatic period versus 378.4 $\mu\text{mol/L}$ in the control group). We believed that the cause of the OS was not completely eliminated in the SAR group, even during the nonexacerbation period. A larger case series and new oxidant-antioxidant status markers need to be evaluated in more detail in the future.

SH protein types determine 52.9% of the total serum antioxidant efficiency based on studies in healthy persons.⁸ Serum SHs are physiologic free-radical scavengers and regulate antioxidant enzymes relevant to glutathione.¹⁹ There also is an expanding body of evidence that indicates that an unusual SH-SS homeostasis condition is included in the pathogenesis of many types of health problems (Figs. 1 and 2).^{10,11,20–23} Increased OS has been reported in various adult and youth studies of patients with asthma^{6,7,12,14,15,24}; however, there are only a few studies on ROS in SAR and only on nitric oxide.^{16,17} Kalpaklioglu and Kalkan¹⁶ evaluated 141 patients, among whom SAR was diagnosed in 122 (78.2%), non-AR was diagnosed in 34 (21.8%), and 15 subjects were healthy controls. They found that fractional exhaled nitric oxide was significantly higher during the allergen season. Gratzou *et al.*¹⁷ evaluated 23 patients and showed that the nitrate-nitrite and nitric oxide levels were elevated in both patients with SAR and patients with asthma but were significantly higher in the SAR plus asthma group than in the SAR-only group. Our results of reduced antioxidant capacity and increased levels of OS in SAR during the exacerbation period were consistent with the studies by Kalpaklioglu and Kalkan¹⁶ and Gratzou *et al.*¹⁷

The decline in nutritional antioxidant values in allergic diseases has been extensively researched.^{25–27} Allen *et al.*,²⁸ in a review and meta-analysis, investigated 40 studies with respect to vitamins A, C, and E in asthma and concluded that dietary intake of these vitamins was considerably decreased in children and adults with asthma, particularly in those with extreme asthma. Research studies in this field have mainly focused on asthma rather than AR. Our study found that the markers we measured were increased in AR, which thus indicated that OS played a role.

In the studies carried out to date, the SS and SH amounts of low-molecular-weight SS compounds in plasma were calculated by using high-performance fluid chromatography, fluorescence capillary electrophoresis, and bioluminescent systems.⁹ In these complex techniques, splitting procedures, for example, the elimination of residual reductants, along with the precipitation of peptides, are also required. These procedures are time consuming, labor intensive, expensive, and complex. SH biochemistry is a rapidly expanding field, but there are few published manual or automated studies that assessed plasma dynamic SH-SS homeostasis.^{9–11} In this study, we designed a test to determine the dynamic SH-SS homeostasis described by Erel and Neselioglu.⁹ We believe that this novel test will assist future research by being easily available, being relatively inexpensive, and using a manually operated spectrophotometric analysis.

Our study showed that there was elevated OS not only in patients with asthma but also in those with AR compared with healthy controls, which led to the “one airway, one disease” model. A limitation of our study was the low number of patients enrolled. To the best of our knowledge, our study was the first to evaluate the plasma SH levels of oxidant status markers in patients with SAR. It also raised the possibility of treating allergic respiratory diseases by using antioxidant adjunctive therapy as part of a holistic strategy. Chemical compounds that contain SH, such as selenium, alpha lipoic acid, and N-acetyl cysteine, may be used in new studies that concern SAR therapy.^{29,30}

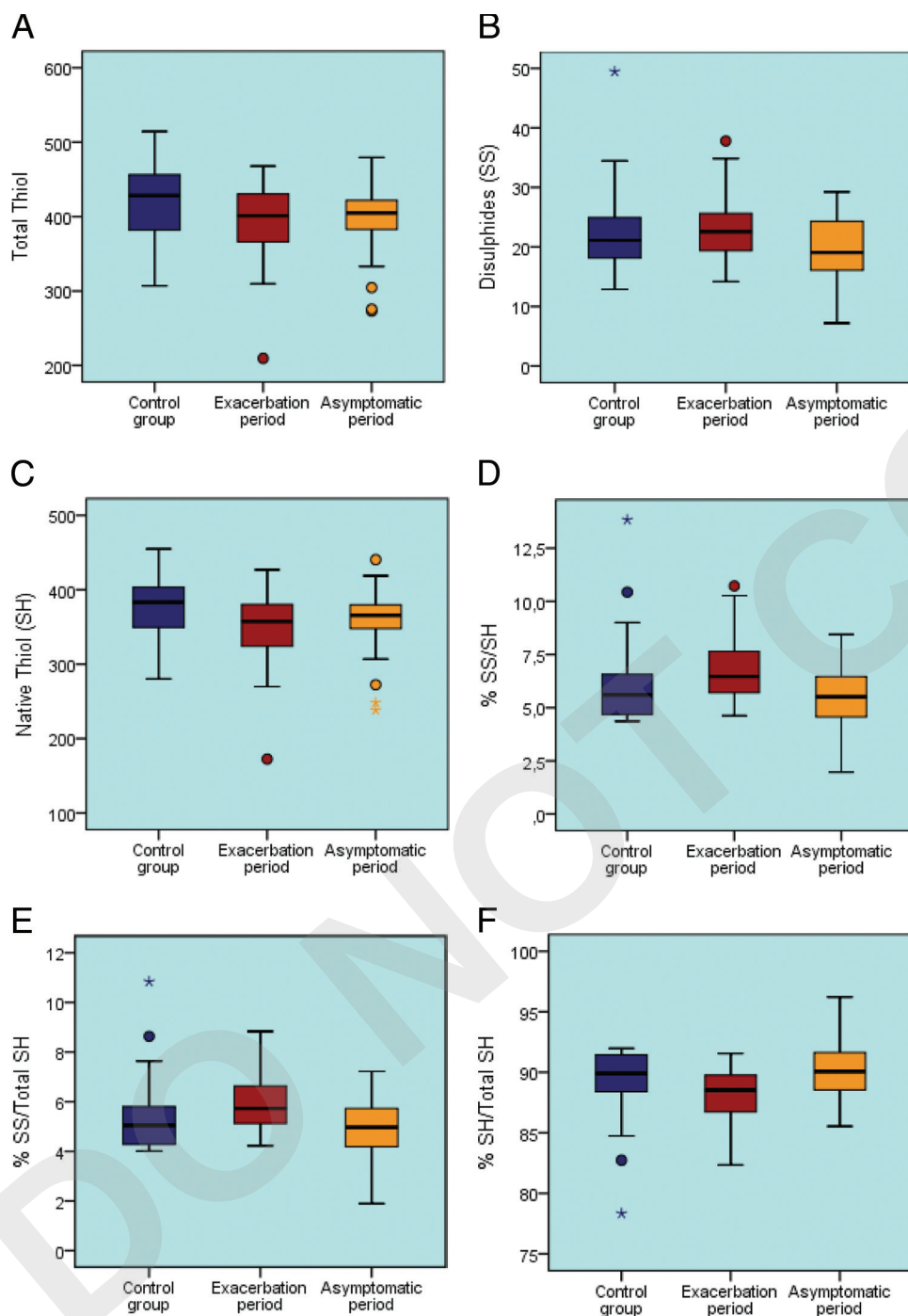


Figure 2. Total Thiol, disulphides (SS), native thiol (SH), % SS/SH, % SS/TT, % SH/TT compared to control group and SAR patients during the exacerbation and asymptomatic periods. % SS/SH = disulphide/thiol percentage ratio; % SS/TT = disulphide/total thiol percentage ratio; % SH/TT = thiol/total thiol percentage ratio.

CONCLUSION

In the exacerbation stage, patients with SAR had increased OS levels and reduced antioxidant enzyme activities; however, in the asymptomatic stage, the levels were reversed. Our results shed light on the etiopathogenesis of the disease and will help in the development of new therapeutic approaches.

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