

# Assessment of Oxidative Stress with Thiol Disulfide Homeostasis and Ischemia-Modified Albumin Level in Acute Urticaria

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## Abstract

**Objective:** There is no study in the literature investigating ischemia-modified albumin (IMA) values and thiol disulfide homeostasis (TDH) parameters in acute urticaria patients. This study aimed assessment of TDH parameters and IMA in acute urticaria patients.

**Methods:** The study included a total of 68 cases, with 35 acute urticaria patients and 33 healthy volunteers. Patients who presented to Ordu University Hospital and were diagnosed with acute urticaria between January 2019 and June 2019 and healthy individuals as the control group were included in the study. Serum albumin, IMA, native thiol, total thiol, and disulfide thiol levels were measured, and the results were compared between the groups.

**Results:** IMA values of  $0.93 \pm 0.09$  in the study group were significantly high compared to  $0.8 \pm 0.10$  in the control group ( $p < 0.01$ ). Native thiol (SH) level was  $353.66 \pm 87.5$  in the study group,  $393.62 \pm 47.7$  in the control group ( $p: 0.022$ ), and total thiol (TSH) level was  $385.46 \pm 86.6$  in the study group and  $433.53 \pm 56.06$  in the control group ( $p: 0.008$ ). In the patient group there was a significant negative correlation between SH levels and IMA levels ( $r = -0.626$ ,  $p < 0.001$ ).

**Conclusion:** In acute urticaria, IMA increases while SH and TSH levels reduce. However, TDH does not change. The lack of change in the balance may be explained by acute urticaria being an acute event and not being a chronic inflammatory process.

**Key words:** acute urticaria, thiol disulfide, ischemia-modified albumin, oxidative stress

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## Introduction

Urticaria is a disease occurring as itchy and edematous papules/plaques called urtica, with

angioedema (AE) linked to deep dermis or subcutis involvement or development of one or the other. Clinical tableau lasting less than six weeks is called acute urticaria (AU), while clinical tableau lasting six weeks or longer is called chronic urticaria (CU). Nearly 15-20% of people are determined to experience one AU attack during a period of their lives (1).

The most common triggers of AU attacks are infections, medications, and foods; however, in 50% of patients the situation remains idiopathic. Detailed history and physical examination are required for assessment of AU patients (2).

The balance between oxidants and antioxidants in the organism is the basis of preserving cellular and biochemical functions. Oxidants damage lipids, proteins and DNA in cells and even cause death (3,4). The most common and rapidly affected proteins are thiols containing sulfhydryl. Plasma thiols are strong antioxidants physiologically removing free radicals (ROS). Serum levels of plasma thiols are counted among markers showing antioxidant levels in the body (5). Normally there is a balance between thiols and disulfides, and these play a protective role for cellular redox homeostasis. This is called dynamic thiol/disulfide homeostasis. Defects in this balance may be associated with a variety of diseases (6). There is increasing evidence that ROSs play a role in the pathogenesis of a variety of inflammatory and allergic diseases including urticaria (7,8).

In recent times, another marker known as an oxidative stress marker is ischemia-modified albumin (IMA). Recent studies have shown ischemia-modified albumin (IMA) levels increase in free oxygen radicals formed because of ischemia, acidosis, or oxidative stress. The most common use is early myocardial injury. There are studies showing IMA levels increase in diseases causing oxidative stress like non-cardiac hypoxia, chronic renal disease, hypercholesterolemia, systemic sclerosis, and type 2 diabetes mellitus. In recent years, high levels of IMA are associated with a variety of diseases linked to oxidative stress (9).

There are very few studies about the correlation with oxidative stress in urticaria (4,10). There are a variety of studies reporting contradictory information about the oxidative stress status of chronic idiopathic urticaria patients. However, the number of studies investigating the thiol disulfide homeostasis in urticaria is very low and the majority are associated with chronic urticaria (4,10-12). There is no study in the literature investigating IMA values and TDH parameters in acute urticaria patients. In this sense, our study is the first to investigate IMA and TDH

parameters together in acute urticaria patients. With this aim, the new oxidative stress markers of thiol disulfide homeostasis and ischemia-modified albumin and the place of oxidative stress in acute urticaria pathogenesis was investigated.

### Methods

The study included a total of 68 cases, with 35 being acute urticaria patients and 33 healthy volunteers. Patients who presented to Ordu University Hospital and were diagnosed with acute urticaria between January 2019 and June 2019 and healthy individuals as the control group were included in the study. Patients with physical urticaria and urticarial vasculitis were excluded from the study.

Serum albumin, ischemia-modified albumin, native thiol, total thiol and disulfide thiol levels were measured, and the results were compared between the groups. Patients' demographic data such as age and gender, vital signs, symptoms, lesion onset time, and urticaria activity score according to Turkey's urticaria diagnosis and treatment guidelines 2016 were recorded.

### Biochemical analyses

Venous blood samples of cases attending with acute urticaria attack symptoms were taken in smooth gel tubes for biochemical tests (Becton Dickinson and Company, New Jersey, USA) and plasma samples for TDH tests were taken in EDTA tubes (Becton Dickinson and Company, New Jersey, USA). All samples were centrifuged at 1600xg for 10 minutes and stored at -70 °C until study. Biochemical tests were studied with an AU 2700 autoanalyzer (Beckman Coulter Inc. USA) with spectrophotometric methods.

Thiol measurements were assessed with spectrometry (Roche, cobas 501, Mannheim, Germany) using Erel et al.'s "modified Ellman method". Disulfide bonds (-S-S) were broken with sodium borohydride (NaBH<sub>4</sub>) to create free functional thiol groups (-SH). Unused sodium borohydride waste was removed with formaldehyde. In this way, reduction of any disulfide bond created by the DTNB reaction with 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB) was prevented. As a result of the DTNB reaction, total thiol groups were determined as reduced and native thiols. Disulfide parameter is a parameter calculated as half of the SH and TSH content. After SH and TSH were determined, SS, SS/SH+SS %, SH/SH+SS % and SS/SH % were calculated.

Ischemia-modified albumin was analyzed with the albumin cobalt binding (CAB) test. The serum sample had 50 mL 0.1% cobalt (II) chloride added (CoCl<sub>2</sub>, 6H<sub>2</sub>O) (Sigma-Aldrich ChemieGmbH Riedstrasse 2, Steinheim, Germany). After waiting 10 minutes, binding of albumin to cobalt was ensured and 50 mL 1.5 mg/mL dithiothreitol was added. After waiting for the 2-minute incubation period, 1.0 mL 0.9% sodium chloride solution was added. Using a spectrophotometer at 470 nm, the absorbance of samples was measured (Jenway 6315 UV/visible Scanning Spectrophotometers, United Kingdom). Results are given as mg/dL. The albumin concentration was calculated by using the bromocresol green staining method (Biolabo, LesHautesRives, 02160, Maizy, France).

### Statistical analysis

All data analyses were conducted using the SPSS v25 (IBM Inc., Chicago, IL, USA) statistical software package. Prior to the statistical analyses, the data were tested for normality using the Shapiro–Wilks test and for homogeneity of variance using the Levene’s test. Independent samples t-test was used to assess differences between two groups. Crosstabulations were generated to describe the relationship between categorical variables, and the independence check was performed on the crosstabulations using a chi-square test ( $\chi^2$ ) and Contingency Coefficient (CC). Likelihood Ratio Chi-square values (LR  $\chi^2$ ) were calculated for frequencies below 5. The Pearson’s correlation analysis test was used to determine the relation between continuous

variables. All comparisons were two-tailed and P-value less than 5% was considered statistically significant.

### Power analysis

The sample size for this study was estimated by a priori power analysis using GPower 3.1 (Universität Düsseldorf, Düsseldorf) statistical software; assuming a large effect size ( $d=0.80$ ),  $\alpha=0.05$  and  $1-\beta=0.80$ , a minimum sample size of 26 in each group was required to detect the significance of the independent groups in the t-test.

### Ethics of the Study

This study was approved by the local ethic committee of Ordu University Medical Faculty with the 2018/234 numbered decision. All participants were informed in detail about the objective of the study and gave written consent. The study was performed in accordance with the ethical principles of the Declaration of Helsinki.

### Results

A total of 35 patients with urticaria (patient group) and 33 healthy subjects (control group) participated in this study. IMA values were significantly higher in the patient group than in the control group ( $p<0.01$ ). Native thiol (SH), Total thiol values were significantly lower in the patient group than in the control group ( $p<0.01$ ). No other differences regarding study variables were observed between the patient group and the control group ( $p>0.05$ ) (Table 1).

**Table 1.** Descriptive statistics and comparison results for the study variables in the patient and control groups.

	Control (n=33)	Patient (n=35)	P-Value
Albumin	4.476±1.551 (1.5-7.2)	4.559±1.432 (1.7-7.6)	0.819 <sup>NS</sup> (t=-0.230)
IMA	0.848±0.106 (0.6-1.1)	0.936±0.096 (0.7-1.09)	0.001** (t=-3.582)
NATIVE THIOL (SH)	393.621±47.727 (239.8-481.0)	353.669±87.506 (145.5-517.9)	0.022* (t=2.355)
TOTAL THIOL	433.536±56.067 (267.8-549.0)	385.469±86.663 (187.5-549.0)	0.008** (t=2.731)
DISULFIDE (SS)	19.958±7.772 (1.0-36.0)	16.95±3.64 (8.0-23.0)	0.051 <sup>NS</sup> (t=1.99)
SS/SH %	5.052±1.718 (0.2-7.8)	4.916±2.519 (0.9-14.4)	0.797 <sup>NS</sup> (t=-0.259)
SS/TOTAL THIOL %	4.544±1.449 (0.2-6.8)	4.387±1.980 (0.8-11.2)	0.711 <sup>NS</sup> (t=0.371)
SH / TOTAL THIOL %	90.910±2.896 (86.5-99.6)	91.225±3.961 (77.6-98.3)	0.711 <sup>NS</sup> (t=-0.372)

Mean±Standard Deviation (Minimum-Maximum); t, Independent samples t-test.

<sup>NS</sup> Statistically not significant ( $p>0.05$ ); \* Statistically significant ( $p<0.05$ ); \*\* Statistically significant ( $p<0.01$ ).

In the patient group, there was a significant negative correlation between the native thiol (SH) levels and the IMA levels ( $r=-0.626$ ,  $p<0.001$ ). In the patient group, there was a significant positive correlation between the SS/SH (%) levels and the IMA levels ( $r=-$

$0.626$ ,  $p<0.001$ ). Native thiol decreased significantly as IMA increased in the patient group ( $r=-0.684$ ,  $p<0.001$ ;  $r=-0.626$ ,  $p<0.001$ , respectively) (Table 2 and Table 3).

**Table 2.** Pearson correlation coefficients between the study variables of patient and control groups.

		ALBUMIN		NATIVE THIOL (SH)	TOTAL THIOL	DISULFIDE (SS)	SS/SH %	SS/TOTAL THIOL %	SH/TOTAL THIOL %
		IN	IMA						
Albumin	r		-0.232	0.043	0.044	0.009	-0.046	-0.032	0.032
	P		0.181	0.808	0.801	0.960	0.793	0.857	0.855
Ima	r	-0.088		-0.626	-0.620	0.102	0.410	0.409	-0.410
	P	0.627		0.000***	0.000***	0.560	0.014*	0.015*	0.014*
Native Thiol (Sh)	r	0.327	-0.684		0.994	-0.141	-0.693	-0.686	0.687
	P	0.063	0.000***		0.000***	0.418	0.000**	0.000***	0.000***
Total Thiol	r	0.303	-0.665	0.968		-0.029	-0.618	-0.607	0.607
	P	0.087	0.000***	0.000***		0.871	0.000**	0.000***	0.000***
Disulfide (Ss)	r	0.088	-0.298	0.421	0.635		0.716	0.755	-0.755
	P	0.627	0.092	0.015*	0.000***		0.000**	0.000***	0.000***
Ss/Sh %	r	-0.022	-0.088	0.091	0.337	0.937		0.996	-0.996
	P	0.905	0.627	0.616	0.055	0.000**		0.000***	0.000***
Ss/Total Thiol %	r	-0.010	-0.072	0.077	0.324	0.933	0.999		
	P	0.954	0.692	0.671	0.066	0.000**	0.000**		
Sh/Total Thiol	r	0.010	0.072	-0.077	-0.324	-0.933	-0.999	-1.000	
	P	0.956	0.690	0.670	0.066	0.000**	0.000**	0.000***	

r, Pearson correlation coefficient in the patient group; r, Pearson correlation coefficient in the control group.

\*, Statistically significant ( $p<0.05$ ); \*\*\*, Statistically significant ( $p<0.001$ ).

**Table 3.** Pearson correlation coefficients between the study variables of patient and control groups.

	Control (n=33)			Patient (n=35)		
	IMA	NAT. T	TOTAL T	IMA	NAT. T	TOTAL T
NATIVE THIOL (SH)	-			-0.626***		
TOTAL THIOL	0.684***			-0.620***	0.994***	
DISULFIDE (SS)	-	0.968***				
Index 1	0.665***			0.088	-0.192	-0.113
Index 2	-0.298	0.421*	0.635***	0.410*	-0.693***	-0.618***
Index 3	-0.088	0.091	0.337	0.409*	-0.686***	-0.607***
	-0.072	0.077	0.324	-0.410*	0.687***	0.607***
	0.072	-0.077	-0.324			

r, Pearson correlation coefficient

\*,  $p<0.05$ ; \*\*\*,  $p<0.001$

## Discussion

There is a balance between free radical production and the antioxidant system suppressing the increase in ROS in the body. If this balance is disrupted, oxidative stress (OS) occurs. There are studies assessing the oxidative stress in urticaria patients. Some studies have shown an increase in OS, while some studies have chosen no change in OS (13-17).

Most studies researching oxidative stress have been performed on chronic urticaria patients (4,10-12).

Oxidative stress has been studied in acute urticaria (AU) and chronic urticaria patient groups with different markers such as malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and catalase (CAT). Studies by Kalkan et al. compared acute urticaria patients with healthy controls and found Cu/ZN superoxide dismutase (SOD) activities and

MDA levels were high and plasma GSH-PX activity reduced (1,10,14). In allergic diseases, especially asthma and to a lesser degree atopic dermatitis, there is increasing literature about the role of oxidative stress.

Free radicals occurring due to normal metabolism or pathologic processes disrupt the structure and functions of thiol dependent enzymes and cause changes in the thiol/disulfide ratio in the cell environment. Reduced plasma thiol concentration shows increased production of free radicals (6). In this study native thiol and total thiol levels reduced but disulfide thiol levels did not increase. But another oxidant parameter, IMA increased. Reduced native thiol levels are not definitely associated with increasing disulfide levels and similarly increased native thiol levels are not definitely associated with reduced disulfide levels. This may be associated with the immune response of thiols to inflammation. Reduced plasma thiol concentration is proof of free radical production. Reduced thiol levels are also associated with leukocyte activation. Urticaria mast cells are associated with immunologic activation and inflammation with oxidative stress (OS) activation of basophils and eosinophils and finally increased ROS (18). Stimulation of all inflammatory cells produces a significant amount of ROS. Eosinophils have higher peroxidase levels compared to other inflammatory cells and have a unique role in formation of oxidative stress (19).

In the acute urticaria patient group in our study, we identified reduced SH and TSH levels compared to the healthy control group. In the literature there are studies of dynamic thiol/disulfide homeostasis in different diseases. Eren et al. (20) showed total thiols were clearly reduced in migraine patients compared to a control group. Ates et al. (21) showed primary hypertension patients had total disulfide balance moving toward disulfide. Kundi et al. (22) identified reduced native and total thiol levels in patients with acute myocardial infarction compared to the control group and stated that thiol/disulfide homeostasis may be a new oxidative stress (OS) marker for acute myocardial infarction patients. A study by Ozyazıcı et al. (23) stated that the total disulfide balance had slid toward disulfide linked to thiol oxidation in a patient group with acute appendicitis and that this balance may be an OS marker for acute appendicitis patients. Yilmaz et al. (24) reported research on the thiol/disulfide homeostasis in asphalt workers exposed to polycyclic aromatic hydrocarbons.

In ischemic conditions, the shape of the amino terminal tip (N-terminal) of albumin changes and metal binding capacity reduces. As a result, this new

form is a new ischemia marker called ischemia-modified albumin (IMA). Though IMA levels were initially considered specific to ischemia, superoxide radical damage, and exposure to free iron and copper have been shown to cause IMA formation (25). Based on these factors, in addition to ischemia-associated diseases, IMA values were shown to increase in some inflammatory diseases (26-28). In our study, a significant increase was identified in IMA levels in the acute urticaria patients compared to the control group.

Ozdemir et al. (29) reported increased IMA levels in patients with psoriasis due to an adaptive response to oxidative stress and systemic inflammation. However, Erem et al. (30) found the IMA irrelevant to oxidative stress.

Mast cells are known to play an important role in allergic diseases. As a result of antigen exposure, mast cells are sensitized and release many mediators causing allergic symptoms (31). Oxidative stress may ease degranulation of mast cells in response to allergens contributing to the development of allergic reactions. In the literature, there are some studies showing an association between oxidative stress and a variety of allergic diseases (3,13,14,19,31,32). Patella et al. (32) found that hymenoptera venom allergy levels of advanced oxidation protein products were consistently high during immunotherapy. They suggested that an oxidative stress state occurs in patients with hymenoptera allergy.

The colorimetric test performed on serum is based on decreased binding of exogenous cobalt to albumin which occurs in tissue damage caused by free radicals. (33). This issue was supported by the reports suggesting that IMA is strongly related with oxidative stress rather than being a myocardial ischemia marker (34,35).

### Limitations

The lack of follow-up data, single blood sampling and small sample size are the limitations of our study. This study does not allow any conclusion about the causative correlation between acute urticaria and underlying interactions. To clarify the underlying mechanisms, it is necessary to confirm these findings in future studies with larger sample sizes.

### Conclusions

Acute urticaria is a disease without definitely known cause. There are studies related to OS as a part of studies about etiopathogenesis. In our study, our objective data identified that SH and TSH levels reduce, and IMA values increase in AU. In

conclusion, based on our data and literature information, we think increased IMA levels and reduced SH and TSH levels may be markers of oxidative stress in acute urticaria.

**Ethics Committee Approval:** Clinical Studies Ethics Committee of Ordu University, Faculty of Medicine, Decision number: 2018-234 Date: 15 November 2018

**Peer-review:** Externally peer-reviewed.

**Author Contributions:**

**Concept:** A.S. **Design:** A.S., S.O. **Literature Search:** A.S., S.O. **Data Collection and Processing:** I.E.A, S.N, O.E, Y.K.A. **Analysis and/or Interpretation:** I.E.A, S.T.S, S.O. **Y.K.A. Writing:** I.E.A, S.T.S.

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