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Assessment of dynamic thiol/disulfide homeostasis in patients with asthma

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Abstract

Background: Asthma is a chronic inflammatory lung disease and oxidative stress is an important component in airway inflammation. This study aims to investigate dynamic thiol/disulfide homeostasis in patients with asthma.
Methods: A total of 103 subjects, including 56 patients with asthma and 47 healthy controls, of similar age and gender were included in the study. The native thiol, total thiol and disulfide levels and the disulfide-native thiol, disulfide-total thiol and native thiol-total thiol ratios were analyzed and compared between the asthma and control groups using a novel automatized spectrophotometric assay.
Results: The levels of native thiol ($p < 0.001$), total thiol ($p < 0.001$) and disulfide ($p < 0.001$) were significantly lower and the C-reactive protein (CRP) levels ($p < 0.001$) were significantly higher in patients with asthma when compared with those in the control group. A negative correlation was detected between CRP levels and native thiol, total thiol and disulfide levels ($p < 0.05$). A significant positive correlation was detected between forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) levels and native thiol and total thiol levels ($p < 0.01$).
Conclusions: The thiol/disulfide homeostasis parameters may be used as novel oxidative stress markers in asthma but further studies are needed to investigate the role of thiol/disulfide homeostasis in asthma.

Keywords: asthma; oxidative stress; thiol/disulfide homeostasis.

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Introduction

Asthma is a common, chronic inflammatory lung disease and it is characterized by variable and recurring symptoms such as wheezing, breathlessness, chest tightness and coughing, airflow obstruction, bronchial hyperresponsiveness and inflammation [1]. Airway reactivity and airflow limitations are the result of contributions of several cell types and mediators in asthma. High levels of reactive oxygen species (ROS) and reactive nitrogen species and decreased antioxidant defenses play a significant role in airway inflammation and asthma severity [2–7].

The thiol groups of cellular protein are organic compounds containing a sulfhydryl (-SH) group which is one of the protective mechanisms against oxidative cell damage [8]. Thiols (RSH) can undergo an oxidation reaction via oxidants and form disulfide (RSSR) bonds [9]. Disulfide bonds can be reduced to thiol groups and thiol reserves increase again. Through these reactions at the cellular level, dynamic thiol/disulfide homeostasis is maintained [10]. Dynamic thiol/disulfide homeostasis has important roles in antioxidant protection, detoxification, cell signaling and transcription, apoptosis and regulation of enzymatic activity [11, 12]. Thiol/disulfide homeostasis parameters have recently been studied as novel oxidative stress markers related to the pathogenesis of different diseases including cardiovascular diseases [13, 14], diabetes mellitus [15], chronic renal failure [16], autoimmune diseases [17, 18], cancer [19], Alzheimer's disease [20] and fibromyalgia [21]. Dynamic thiol/disulfide homeostasis was initially measured in an easy and repeatable technique in 2014 using a new method developed by Erel and Neşelioğlu [22] with high accuracy and sensitivity.

In this study, we aimed to investigate the levels of native thiol, total thiol and disulfide and the ratios of disulfide/native thiol, disulfide/total thiol and native thiol/total thiol in patients with asthma using a novel, automated method that determines dynamic thiol/disulfide homeostasis.

Materials and methods

A total of 56 (30 females, 26 males) asthmatic patients aged between 18 and 70 years, who were admitted to the Department of Pulmonology, Ahi Evran University Training and Research Hospital between February 2017 and June 2017 and who were previously diagnosed with asthma at least 1 year before without hospitalization within the past 6 months (Group 1), and 47 age- and gender-matched healthy individuals were selected for the study. The diagnosis of asthma in the patient group (Group 1) was considered based on the Global Strategy for Asthma Management and Prevention [Global Initiative for Asthma (GINA) 2017] [23]. Healthy subjects (Group 2) were selected based on normal pulmonary function test (PFT) [forced expired volume in 1 s (FEV1), forced vital capacity (FVC) and peak expiratory flow (PEF) greater than 80%].

Patients with inflammatory diseases, malignancies, diabetes mellitus, cardiovascular diseases, acute chronic kidney or liver diseases were excluded from the study. The study was performed in accordance with the Declaration of Helsinki's Good Clinical Practice guidelines and approved by the Ahi Evran University Ethical Committee (2017-11/104). All subjects provided written informed consent before participation in the study.

Biochemical measurements

Blood samples were obtained following overnight fasting. The collected blood samples were centrifuged at 1500*g* for 10 min to separate the serum. C-reactive protein (CRP) was measured by a immunoturbidimetric method with a commercially available kit using (Roche Diagnostic Corp., Mannheim, Germany) an autoanalyzer. Serum was stored at –80 °C until analysis of thiol/disulfide homeostasis tests.

Thiol/disulfide homeostasis test levels were measured using a newly developed, fully-automated and spectrophotometric method by Erel and Neşelioğlu [22]. After determining native and total thiols, the concentration of disulfide was determined using the formula:

$$\text{Disulfide} = (\text{total thiol} - \text{native thiol}) / 2$$

The ratios of disulfide/total thiol (%), disulfide/native thiol (%) and native thiol/total thiol (%) were calculated using the concentrations of disulfide, native thiol and total thiol, which were previously determined.

Statistical analysis

Analyses were performed using SPSS software (version 16.0, SPSS, Chicago, IL, USA). The Kolmogorov-Smirnov test was used to evaluate the normality of the distributions of variables. Parametric data were expressed as mean ± standard deviation (SD). Student's t-test was used to compare numerical variables with normal distribution. The Mann-Whitney U-test was used to compare numerical variables with abnormal distribution. Spearman and Pearson correlation tests were used to determine the relationship between variables. p-Values <0.05 were considered statistically significant.

Results

The mean age in the asthma patient group (30 females, 53.6%) was 50.2 ± 11.9 years, and the mean age in the control group (23 females, 48.9%) was 48.4 ± 12.8 years. The demographic characteristics including age, gender and body mass index (BMI) were similar in the two groups. Based on the severity of their condition, asthma patients consistently used low or high doses of inhaled corticosteroids, long-acting β_2-agonists, montelukast or shortacting β_2-agonists. Healthy controls did not use any medications. The results of the respiratory function test of asthmatic patients were: FEV1% was 67.8 ± 19.7; FVC% was 75.9 ± 16.5; the ratio of FEV1/FVC was 71.4 ± 10.3; the PEF rate was 54.2 ± 20.1.

The CRP levels in the asthma patient group were statistically higher ($p < 0.001$, Table 1). The thiol/disulfide homeostasis parameters including the levels of native thiols, total thiols and disulfide were statistically lower in the asthma patient group ($p < 0.001$, Table 1). The demographic features and laboratory findings of the study population are shown in detail in Table 1.

Table 1: Demographic features and laboratory findings of the study population.

Variables	Asthma group (n = 56)	Control group (n = 47)	p-Value
Gender (female), n (%)	30 (53.6)	23 (48.9)	0.639
Age, year	50.2 ± 11.9	48.4 ± 12.8	0.34
BMI, kg/m^2	28.1 ± 3.1	27.4 ± 2.7	0.217
CRP, mg/dL	0.78 ± 0.6	0.2 ± 0.2	<0.001[a]
Native thiol, μmol/L	231.2 ± 54.4	343.6 ± 125.3	<0.001[a]
Total thiol, μmol/L	365.5 ± 65.9	561.4 ± 119.8	<0.001[a]
Disulfide, μmol/L	67.1 ± 11.1	108.9 ± 21.2	<0.001[a]
Disulfide/native thiol, %	30.4 ± 9.4	39.8 ± 27.2	0.123
Disulfide/total thiol, %	18.6 ± 2.8	20.3 ± 6.3	0.124
Native thiol/total thiol, %	62.8 ± 5.7	59.3 ± 12.7	0.122

BMI, body mass index; CRP, C-reactive protein; parameters were expressed as mean ± standard deviation (SD). [a]$p < 0.05$ was considered significant for statistical analyses.

The correlation analysis of native thiol, total thiol, disulfide, disulfide/native thiol, disulfide/total thiol and native thiol/total thiol with demographic and clinical findings of asthma patients is shown in detail in Table 2. The correlation analysis showed a significant negative correlation of age with native thiol, total thiol, disulfide and native thiol/total thiol parameters and a significant positive correlation with disulfide/total thiol parameter (Table 2, $p<0.05$). The CRP level displayed a negative correlation with native thiol, total thiol, disulfide and disulfide/total thiol levels, and a positive correlation with disulfide/native thiol and native thiol/total thiol levels (Table 2, $p<0.05$, Figure 1). The BMI showed a significantly negative correlation only with native thiol/total thiol levels of the thiol/disulfide parameters. The FEV1 and FVC parameters showed a significantly positive correlation with native thiol and total thiol levels (Table 2, $p<0.01$) (Figures 2 and 3). FEV1/FVC measurements did not show any significant correlation with any of the thiol/disulfide homeostasis parameters (Table 2, $p>0.05$). The PEF showed a significantly positive correlation only with disulfide levels of the thiol/disulfide parameters. When the relationship between thiol/disulfide parameter levels and drugs were analyzed, there was no significant correlation ($p>0.05$).

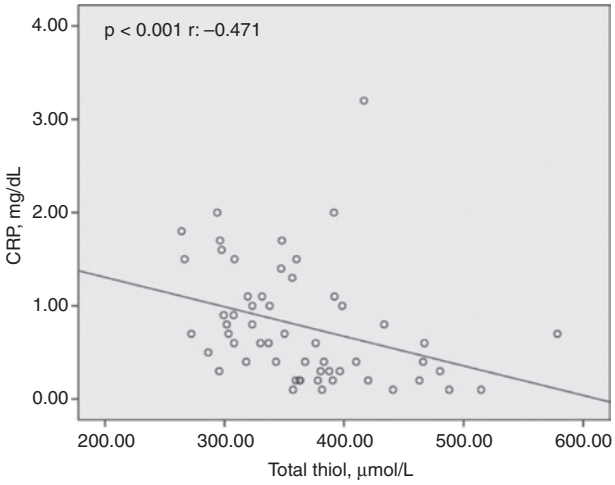

Figure 1: The correlation analysis of C-reactive protein (CRP) and total thiol.

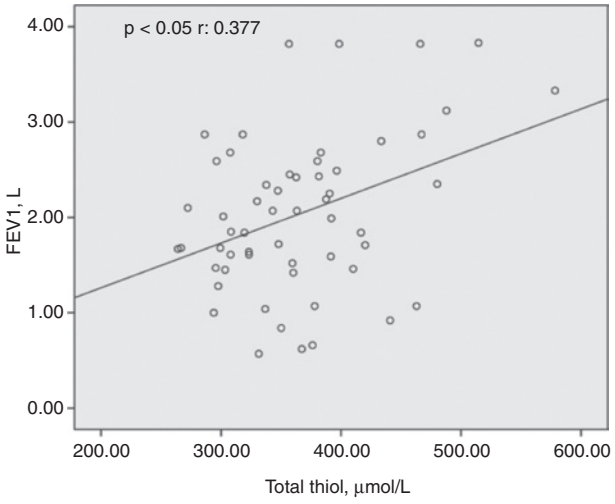

Figure 2: The correlation analysis of FEV1 and total thiol.

Discussion

Asthma is the most common chronic inflammatory lung disease and oxidative stress is an important component in airway inflammation. Increased free radicals such as ROC and reactive nitrogen species and the deficiency of free radical-scavenging antioxidants are important in

Table 2: The correlation coefficients between demographic features/laboratory findings of the asthma patients and thiol/disulfide homeostasis parameters.

Variables	Native thiol		Total thiol		Disulfide		Disulfide/total thiol		Disulfide/native thiol		Native thiol/total thiol	
	r	p-Value	r	p-Value	r	p-Value	r	p-Value	r	p-Value	r	p-Value
Age, year	−0.579	<0.001[a]	−0.589	<0.001[a]	−0.332	0.013[a]	0.295	0.027[a]	0.260	0.053	−0.295	0.028[a]
BMI, kg/m^2	−0.054	0.693	−0.065	0.635	−0.061	0.658	0.012	0.930	0.015	0.911	−0.012	0.047[a]
CRP, mg/dL	−0.495	<0.001[a]	−0.471	<0.001[a]	−0.244	0.07	−0.450	0.001[a]	0.450	0.001[a]	0.450	0.001[a]
FEV1	0.350	0.008[a]	0.377	0.004[a]	0.260	0.053	−0.137	0.315	−0.110	0.419	0.137	0.315
FVC	0.473	<0.001[a]	0.461	<0.001[a]	0.211	0.118	−0.279	0.037[a]	−0.231	0.087	0.279	0.037[a]
FEV1/FVC	−0.130	0.340	−0.056	0.683	0.152	0.264	0.241	0.074	0.221	0.102	−0.241	0.074
PEF	0.152	0.274	0.227	0.099	0.309	0.023[a]	0.052	0.707	0.076	0.585	−0.052	0.707

BMI, body mass index; CRP, C-reactive protein; FVC, forced vital capacity; FEV1, forced expired volume in 1 s; PEF, peak expiratory flow.
[a]$p<0.05$ was considered significant for statistical analyses.

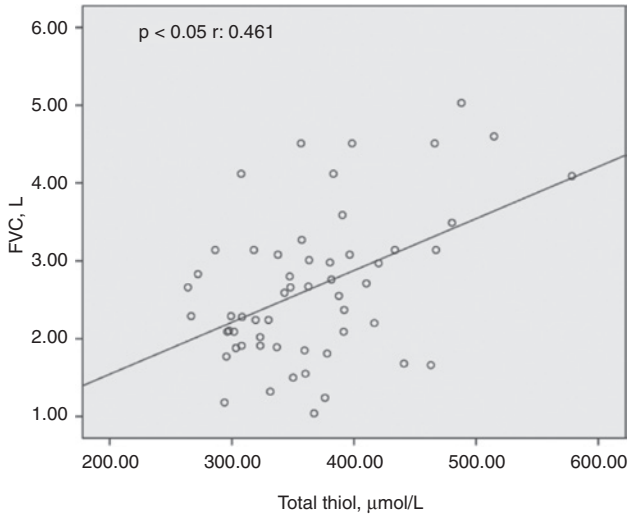

Figure 3: The correlation analysis of FVC and total thiol.

asthma etiopathogenesis [24, 25]. Thiols are an important component of the plasma antioxidant system and are functional sulfhydryl groups, which consist of a sulfur atom and a hydrogen atom bound to a carbon atom [26]. Mainly albumin, glutathione (GSH), thioredoxin, cysteine and homocysteine constitute the plasma thiol pool [27]. Determination of dynamic thiol/disulfide homeostasis can provide valuable information on various biochemical processes [22]. In the current study, we hypothesized that there might be impaired thiol/disulfide homeostasis in asthma patients. The results from our study demonstrated that in asthma patients, some of the thiol/disulfide homeostasis parameter levels were different compared to those observed in healthy controls, and showed a significant correlation with age, CRP and PFTs. To the best of our knowledge, there is not much study to show that thiol/disulfide homeostasis may play a role in asthma patients.

There are limited data regarding the determination of the oxidant-antioxidant status in asthma patients to show that oxidant-antioxidant imbalance is related to the etiopathogenesis and severity of asthma. These studies evaluated oxidant and antioxidant molecules such as malondialdehyde (MDA), superoxide dismutases (SOD), GSH peroxidases (Gpx), melatonin (MEL), total protein sulfhydryl levels and total antioxidant capacity [28–31]. Rahman et al. [32] reported a decreased plasma total antioxidant capacity in asthmatic patients. Gumral et al. [33] compared the antioxidant defense mechanism in patients with bronchial asthma and healthy controls and they demonstrated that the serum MEL levels and the erythrocyte SOD activity in patients with bronchial asthma were significantly lower than in the healthy group and these results provide some evidence for the potential role of decreased antioxidant enzymes, MEL and respiratory function test values in asthma. Shokry and El-Tarahony [28] found significantly low SOD and Gpx activities and high MDA in asthmatic children. Ahmad et al. [31] and Nadeem et al. [34] showed decreased plasma total protein sulfhydryl levels and total antioxidant capacity in asthma patients compared to the healthy controls.

Another important point in our study is that the FEV1 and FVC parameters showed a significantly positive correlation with native thiol and total thiol levels ($p < 0.001$). The decreased antioxidant defense may be a reason for the increased airway obstruction and disease severity [31]. There are few studies in the literature that demonstrated the antioxidant status of asthma and the correlation between PFTs however, these studies are consistent with our results. Ahmad et al. [31] also found a positive association with FEV1 and total antioxidant status, erythrocyte GPx and total protein sulfhydryls. They concluded that oxidant-antioxidant imbalance plays a significant role in the severity of the disease.

In the study by Dilek et al. [35], the plasma total thiol (PTT) levels and total antioxidant status were found to be reduced in asthmatic children. The study also showed that montelukast-treated asthmatic patients had PTT levels similar to the control group. They reported that montelukast which decreases ROS production in whole blood can limit oxidative stress [36] and restore depleted PTT levels [35]. In our study, there was no relationship between thiol/disulfide parameter levels and drugs. In another study by Kaya et al. [37], native thiols, total thiols and disulfide median levels were higher in asthmatic children compared to the healthy controls. They reported that the disulfide/thiol ratio and disulfide levels were significantly higher in the asthmatic group than in the control group ($p < 0.05$) [37]. Our findings showed that native thiols, total thiols and disulfide levels were statistically lower in the asthma patient group compared to the control group ($p < 0.0015$, Table 1).

Babaoglu et al. [30] compared the parameters related to thiol/disulfide homeostasis in patients with chronic obstructive pulmonary disease (COPD), asthma and asthma-COPD overlap syndrome (ACOS). The findings in the thiol/disulfide homeostasis parameters were similar in the three patient groups ($p > 0.05$). They concluded that COPD, asthma and ACOS share similar pathophysiological features but display different clinical manifestations [30].

The disulfide level is expected to increase and the thiol level decreases under oxidative stress. But the disulfide levels observed in our study were lower than those in healthy subjects. These findings may be explained by the

factors affecting the thiol-disulfide balance except oxidative stress such as the rates of the thiol-disulfide exchange reactions, thiol oxidation by ROS and possible repair processes, enzymatic extracellular degradation of GSH and liver release of thiol-containing molecules [27, 38].

Serum CRP levels which are known to be a marker of systemic inflammation were significantly higher in asthma patients compared to the control group (Table 1, $p < 0.001$). This result shows us the role of inflammation in the pathophysiology of asthma and it is consistent with studies in the literature that examine the association of asthma and CRP. Fujita et al. [39] showed that the mean serum CRP levels were significantly higher in asthma patients with and without attacks than in controls ($p < 0.001$). In the study by Babaoglu et al. [30], the native thiol and total thiol levels were significantly and negatively correlated with the CRP levels in patients with COPD, asthma and ACOS ($p < 0.05$). Also, consistent with this study, the CRP levels were significantly negatively correlated with native thiol, total thiol and disulfide measurements in asthma patients in our study (Table 2, $p < 0.05$).

Study limitations

A major limitation of our study was the small sample size of the study population. A larger study population with long-term follow-up of patients may offer more powerful statistical data. Although additional oxidant and antioxidant markers were not measured in our study, their investigation would be a worthwhile area of research. Another limitation is the lack of use of this new method in the assessment of asthma and it was limiting for comparing the results with other studies.

Conclusions

This study shows that the levels of native thiol, total thiol and disulfide decreased significantly in patients with asthma. Moreover, these parameters were found to be correlated with PFTs. These results suggest that the thiol/disulfide homeostasis parameters can be helpful to understand the physiopathology of asthma and may be a new biomarker in asthma; however, further larger-scale prospective studies would help to validate our findings.

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