



Original Contribution

**LIPID PEROXIDATION AND CATALASE ACTIVITIES IN PATIENTS WITH
CHRONIC OBSTRUCTIVE PULMONARY DISEASE:
A COMPARATIVE STUDY WITH OTHER PULMONARY DISEASES**

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is one of the most common chronic diseases and a major cause of morbidity and mortality. An imbalance between oxidants and antioxidants (oxidative stress) has been proposed as a critical event in the pathogenesis of COPD. The aim of the present study was to find whether an oxidative stress occurs at the clinical onset of COPD and some other pulmonary diseases through the level of lipid peroxidation products in plasma and the activity of CAT an important extracellular antioxidant enzyme. We evaluated a biomarker of oxidative stress malondialdehyde, a lipid peroxidation derived product (MDA) and an enzymatic antioxidant catalase (CAT) in COPD patients and healthy controls. The marker of oxidative stress was found to be significantly ($p < 0.001$) higher in COPD patients when compared with control group. COPD patients had a significant ($p < 0.0001$) increase in antioxidant enzyme CAT as compared with control group. Our results show that oxidative stress is an important pathophysiologic change in COPD.

Key words: Chronic obstructive pulmonary disease, Oxidative stress, Lipid peroxidation, Antioxidants, Catalase.

INTRODUCTION

Low concentration of reactive oxygen species (ROS) such as O_2 , H_2O_2 , OH may be beneficial or even indispensable in processes such as intracellular messaging and defense against micro-organisms, contributing to phagocytic bactericidal activity. In contrast, high doses and/or inadequate removal of active oxygen results in oxidative stress, which may cause severe metabolic malfunctions. ROS lead to lipid peroxidation, specific oxidation of some enzymes, oxidation of proteins and nucleic acids and their degradation. The oxidative stress and oxidative injuries are considered to be a component of virtually every disease process. Chronic obstructive pulmonary disease (COPD) is one of the most common chronic diseases and

represents an important cause of morbidity and mortality. COPD is inflammatory lung diseases that is characterized by systemic and chronic localized inflammation and oxidative stress (1). Sources of oxidative stress arise from the increased burden of inhaled oxidants, as well as elevated amounts of reactive oxygen species (ROS) released from inflammatory cells. Increased levels of ROS, either directly or via the formation of lipid peroxidation products, may play a role in enhancing the inflammatory response in COPD (2). Moreover, in COPD it is now recognized as the main pathogenic factor for driving disease progression and increasing severity. The involvement of oxidative stress in the pathogenesis of COPD appears to be crucial for the manifestation of the inflammatory response of the lung (3). ROS and lipid peroxidation products can influence the inflammatory response at many levels through its impact on signal transduction mechanisms, activation of redox-sensitive transcriptions

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factors, and chromatin regulation resulting in pro-inflammatory gene expression that lead to the poor efficacy of corticosteroids in COPD.

Thus, the presence of oxidative stress has important consequences for the pathogenesis, severity, and treatment of COPD. However, for ROS to have such an impact, it must first overcome a variety of antioxidant defenses.

Erythrocytes are excellently equipped to handle intracellular oxidative stress through the combined activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione (GSH). However, the protective efficiency of erythrocytes against lipid peroxidation would depend on the balance between oxidant species and the availability of antioxidant defenses. Experimental studies have provided evidence about an imbalance between oxidants/antioxidants, in favor of reactive oxidizing species (oxidative stress), associated with COPD (4,5).

Therefore, the aim of the present study was to find whether an oxidative stress occurs at the clinical onset of COPD and some other pulmonary diseases through the level of lipid peroxidation products in plasma and the activity of CAT an important extracellular antioxidant enzyme.

MATERIAL AND METHODS

Patients

This study was carried out on 18 patients with pulmonary diseases ranged in age from 19 to 78 from the Department of Internal Medicine, University Hospital, Medical Faculty, Stara Zagora. A group of 20 healthy aged – matched volunteers of either sex were chosen as a control group. Patients were not treated with drugs. All of the patients were hospitalized because of acute infection of lower respiratory tract.

Blood Samples and Lysates

Blood was collected in tubes containing ethylenediamine-tetraacetic acid (EDTA), centrifuged at 3000 rpm for 15 min and plasma was carefully separated. After the erythrocyte pellet was washed three times with saline, and 0.5 ml of the cell suspension was diluted with 2 ml cold water to lyse the erythrocytes. To 0.2 ml lysate 1.8 ml water and ethanol/ chloroform (3:5/v:v) were then added to precipitate hemoglobin. The tubes were shaken vigorously for 5 min and centrifuged at 2500 rpm for 20

min. The supernatant was used for determination of enzyme activity.

Quantification of Lipid Peroxidation

Total amount of lipid peroxidation products in the plasma of healthy volunteers and patients was estimated using the thiobarbituric acid (TBA) method, which measures the malondialdehyde (MDA) reactive products (6). In brief, 1.0 ml of plasma, 1.0 ml of normal saline and 1.0 ml of 25% trichloro-acetic acid (TCA) were mixed and centrifuged at 2000 for 20 min. One ml of protein free supernatant was taken, mixed with 0.25 ml of 1% TBA and boiled for 1 h at 95° C. After cooling, the intensity of the pink color of the obtained fraction product was read at 532 nm. Results were expressed as $\mu\text{M/l}$.

Monitoring of Catalase Activity

CAT activity was assessed in the erythrocyte lysates by the method described by Beers and Sizer (7). Briefly, hydrogen peroxide (30 mM) was used as a substrate and the decrease in H_2O_2 concentration at 22°C in phosphate buffer (50 mM, pH 7.0) was followed spectroscopically at 240 nm. One unit of CAT activity is defined as the amount of enzyme that degrades 1 μM H_2O_2 per min. Results are presented as units per mg hemoglobin (U/g Hb).

Statistical Methods

The results are reported as means \pm SD for the patients and the control group. Statistical analysis was performed with Student's t-test and Multiple regression analysis. $p < 0.05$ was considered statistically significant.

RESULTS

Figure 1 shows the marker of oxidative stress evaluated as the amount of MDA produced in COPD patients compared with other pulmonary diseases such as asthma, pneumonia etc.. The MDA plasma levels were found to be significantly higher in COPD patients when compared with the control group (mean 2.12 $\mu\text{M/l}$ vs 1.52 $\mu\text{M/l}$, $p < 0.001$). We did not find significant differences in MDA levels compared with the control group in patients with asthma, pneumonia and other pulmonary diseases group (mean 1.88 $\mu\text{M/l}$; 1.99 $\mu\text{M/l}$ and 1.91 $\mu\text{M/l}$, $p > 0.05$) There was not significant difference in MDA levels among the asthma, pneumonia and other pulmonary diseases groups.

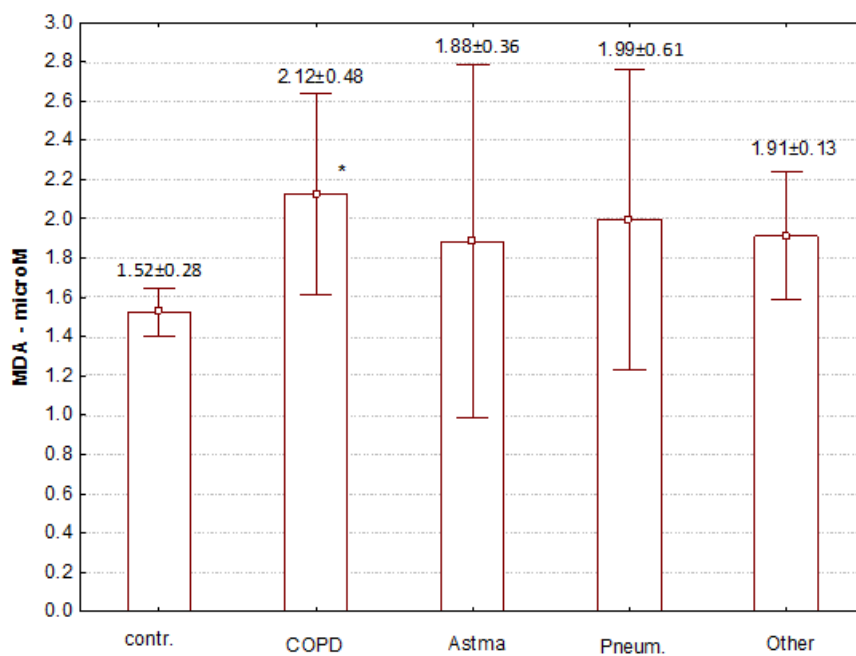


Figure 1. MDA in patients with COPD; *p<0.001 compared to controls

COPD patients had a significant increase also in antioxidant status (CAT activity) when compared with the control group (mean 50118.10 U/gHb vs 17157.75U/gHb, p<0.0001) (**Figure 2**). In patients with asthma, pneumonia and other pulmonary diseases the activity of this

enzyme was not significantly changed compared to that of the controls (mean 36204.97 U/gHb, 37181.05U/gHb, and 34 911,80U/gHb, p>0.05). There was not significant difference in CAT activities among the asthma, pneumonia and other pulmonary diseases groups.

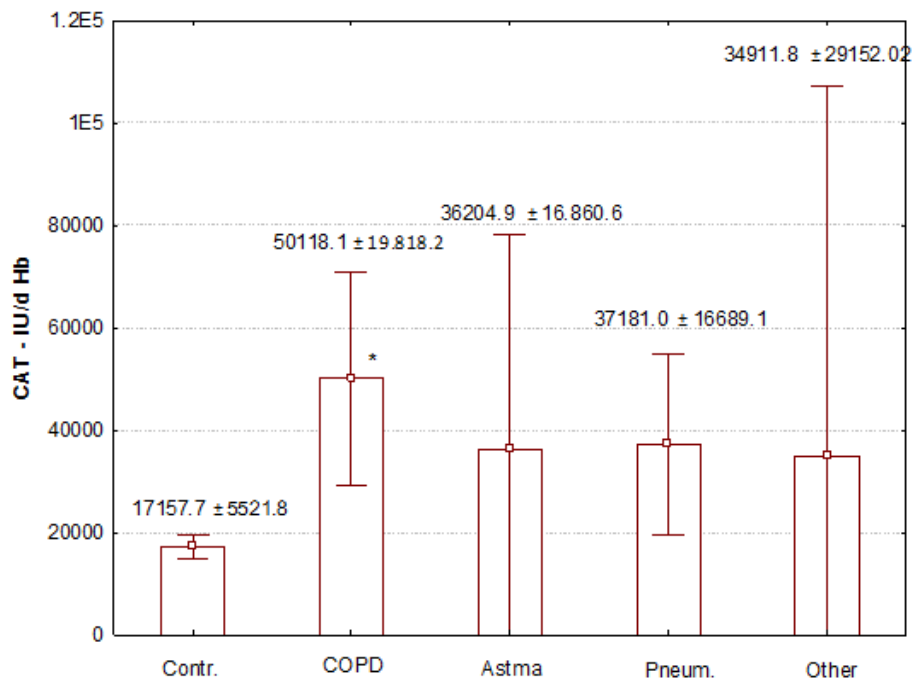


Figure 2. CAT in patients with COPD; *p<0.0001 compared to controls

The correlation between MDA level and CAT activity in patients with COPD is shown on **Figure 3**. Well expressed positive correlation was found for COPD ($R=0.72$, $p=0.005$,

Regression analysis), but not for asthma, pneumonia and other pulmonary diseases groups.

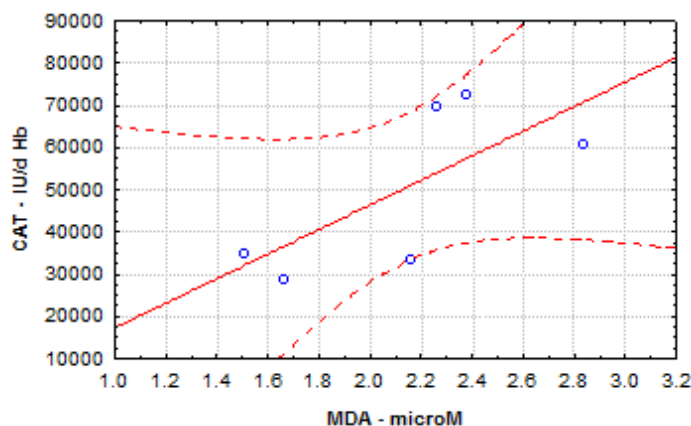


Figure 3. Correlation between MDA level and CAT activity in patients with COPD; $R=0.72$, $p=0.005$, (Regression analysis).

DISCUSSION

The lung is the organ with the highest exposure to ambient air in the entire human architecture. Due to its large surface area and blood supply, the lung is susceptible to oxidative injury in the form of reactive oxygen species (ROS) and free radicals. In order to provide defense against the oxidative burden, the lungs produce various endogenous agents called antioxidants. There is plenty of evidence supporting an imbalance between oxidants and antioxidants in the lung of COPD patients (4,5). The causes of increased oxidative stress and alterations of antioxidant enzymes in patients with COPD have not been completely explained. For this purpose we investigated and compared the level of lipid peroxidation products (MDA), a useful tool to show the occurrence of an oxidative stress, and the activities of CAT an important extracellular antioxidant, in patients with COPD. A comparison was made with patients with asthma, pneumonia and other kind of pulmonary diseases.

Patients with COPD appeared to had increased level of lipid peroxidation products compared to the healthy volunteers and those with asthma, pneumonia and other kind of pulmonary diseases. These findings are in line with the result of other studies describing enhanced level

of oxidative damages in asthma and COPD patients (8, 9). In the current study, based on the increased lipid peroxidation products detected in patients with COPD, occurrence of oxidative stress in these patients was suggested. The increase in the lipid peroxidation products in plasma of patients with COPD, supports the hypothesis of oxidative stress associated with the disease.

Antioxidants are also markers of oxidative stress. Even though CAT is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells (10). This suggests the pivotal role of catalase for cell adaptation to oxidative stress (11). In the present study, CAT activity was significantly higher in patients with COPD, compared to the control group and those with asthma, pneumonia and other kind of pulmonary diseases. The activation of erythrocyte catalase, could contribute to the detoxication of reactive oxygen species. In addition, we observed a direct positive correlation between erythrocyte CAT levels and plasma MDA levels in patients with COPD. This relationship focus on the additional protective response to the continuous oxidant stress present in these patients. Other studies showed increased oxidative stress and

altered levels of antioxidants in patients with COPD as compared to control subjects (12). However discrepant results were found in other studies regarding the relationship between antioxidant status and pulmonary functions in COPD patients

In our study oxygen therapy was not analyzed. However the effect of this therapy in oxidative stress in COPD patients is still controversial. Further studies are required to clarify the effects of oxygen therapy in COPD patients. It is likely, therefore, that a combination of antioxidants may be effective in the treatment of asthma and COPD (13).

CONCLUSIONS

- An increased oxidative stress present in patients with COPD, demonstrated by the increased lipid peroxidation (MDA) in the plasma of these patients could be a possible criteria for the inflammation of this disease.
- The activity of the antioxidant defense enzyme CAT in patients with COPD is also higher than that of the controls that could be a possible criteria for the additional protective response to the continuous oxidant stress present in these patients.
- A positive correlation existed between MDA level and CAT activity in COPD patients group but not in patients groups with asthma, pneumonia and other pulmonary diseases.

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