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Activity of the antioxidant system enzymes in patients with different clinical forms of the pulmonary tuberculosis

Abstract: This article is devoted to the activity of the antioxidant system enzymes in patients with different clinical forms of the pulmonary tuberculosis. The publication focuses on the dependence of the extent of the damage to the AOS enzyme components on the clinical form of tuberculosis and the correlation of the inhibition of the AOS enzymes' activity with the level of carbonyl groups in the blood plasma before and after the intensive phase of the treatment as well as the comparison of the AOS enzymes' activity of the patients with tuberculosis compared to that of the healthy individuals.

Keywords: tuberculosis, infiltrative form, disseminated form, oxidative stress, reactive oxygen species, antioxidant system enzymes, carbonyl groups.

Introduction

The patients with tuberculosis have got an imbalance between the production of reactive oxygen species (ROS) in the lung tissues affected by the tuberculous process and the activity of the antioxidant defense system [1, 2].

Considering the complex mechanisms of the tuberculosis infection pathogenesis, the study the activity of the various components of the antioxidant system (AOS) in conjunction with the clinical course of the disease is of great interest.

The purpose of the study was the research of AOS enzymes' activity: the Cu/Zn-superoxide dismutase (SOD1), the Mn-superoxide dismutase (SOD2), the catalase (Cat), and the glutathione-dependent enzyme system: the glutathione peroxidase (GPx), the glutathione reductase (GRed), the glutathione-S-transferase P1 (GSTP1) as well as the levels of carbonyl groups in the peripheral blood of patients with pulmonary tuberculosis before and after the intensive phase of the treatment, depending on the clinical variants of the tuberculous process.

Materials and methods

The AOS activity was determined in 83 patients with tuberculosis, aged 21 to 74 years old, admitted for treatment in the Odessa Regional TB Clinical Hospital before and after two months of the standard treatment (the intensive phase). The control group consisted of 23 healthy people. The study was conducted in accordance with the agreement between the Odessa National Medical University and Odessa Regional TB Clinical Hospital. All the subjects were informed about the aims and objectives of the upcoming study and gave given informed consent for their participation in the study. The research results were analyzed in the total group of patients, as well as groups with different forms of tuberculosis (infiltrative and disseminated). All the aforementioned AOS components were evaluated in the peripheral blood. The samples were centrifuged at 3000 rev/min in order to separate the plasma and packed cells.

The activity of SOD1 and SOD2 [3, 4], Cat [5], GSTP1 [6, 7], GPx, GRed [8, 9] was determined in the blood cells, while the level of carbonyl groups was determined in the plasma [10]. The protein level was determined via the Lowry method [11, 12].

The results were analyzed statistically via the Microsoft Excel 2013 program. The degree of difference was calculated with via the Student t-test. The correlation (r) between the individual AOS components was calculated as well.

Results

The activity of all the studied enzymes in patients with tuberculosis was significantly reduced compared to the control group of healthy individuals (Table 1).

The most pronounced inhibition of the activity was typical for SOD1, SOD2, GPx and GRed (more than 30% compared to those of the control group (100%)). The reduction of enzyme activity was observed alongside the significant increase of the level of carbonyl groups in the plasma (up to 143% compared to the control group - 100%).

After two months of treatment there was a statistically significant increase in the activity of all the investigated components of the AOS, which however still remained low compared to the control group. The level of carbonyl groups decreased as well, but it did not reach the corresponding value of the control group.

Table 1. Activity of the AOS enzymes in tuberculosis patients

Researched values	Healthy	Tuberculosis patients (n=83)	
	individuals (n=53)	Before treatment	After treatment
	M±m	M±m	M±m
SOD1 (unit/ml)	32,5±2,7	21,95±0,51*	27,46±0,62**
SOD2 (unit/ml)	21,3±1,5	14,65±0,36*	18,12±0,37*..
GSTP1 (mccat/l)	326,2±21,4	254,72±3,87*	284,37±4,13**
GPx (mccat/l)	213,4±14,8	138,15±1,78*	150,58±2,54*..
Cat (mccat/l)	38,6±2,7	28,88±0,33*	31,51±0,47*..
GRed (mccat/l)	24,8±1,9	14,97±0,22*	17,91±0,86*..
Carbonyl groups (nmol/mg of protein)	63,2±5,3	90,46±1,79*	84,78±1,91*..

* – significant difference between the groups of tuberculosis patients and healthy individuals- (p<0,05)

** – significant difference compared with the baseline (before treatment) - (p<0,05)

The reduction of the antioxidant enzyme activity correlated with the increase of the level of carbonyl groups (Table 2).

Table 2. Correlation between the level of carbonyl groups and the activity of the AOS enzymes in tuberculosis patients

R-value (carbonyl groups/enzyme)	Tuberculosis patients (n=83)	
	Before treatment	After treatment
Enzyme		
SOD1	-0,572*	-0,039
SOD2	-0,598*	-0,270
Cat	-0,844*	-0,183
GSTP1	-0,569*	-0,330*
GRed	-0,763*	-0,383*
GPx	-0,892*	-0,424*

* – $p < 0,05$

After the treatment the enzyme activity is significantly improved compared to the baseline in both groups. In some cases (GSTP1 – in both groups, SOD1 – the group with the infiltrative form, SOD2 – the group with the disseminated form) the differences with the control group were not significant statistically-wise ($p > 0,05$). Furthermore, there was also no difference found between these groups regarding the activities of SOD2, GSTP1 and GRed ($p > 0,05$).

After two months of treatment the negative correlation persisted in all the cases except SOD1. In the patients with the disseminated form of tuberculosis the same pattern was observed before the treatment, but afterwards only GPx had a weak negative correlation with the level of carbonyl groups.

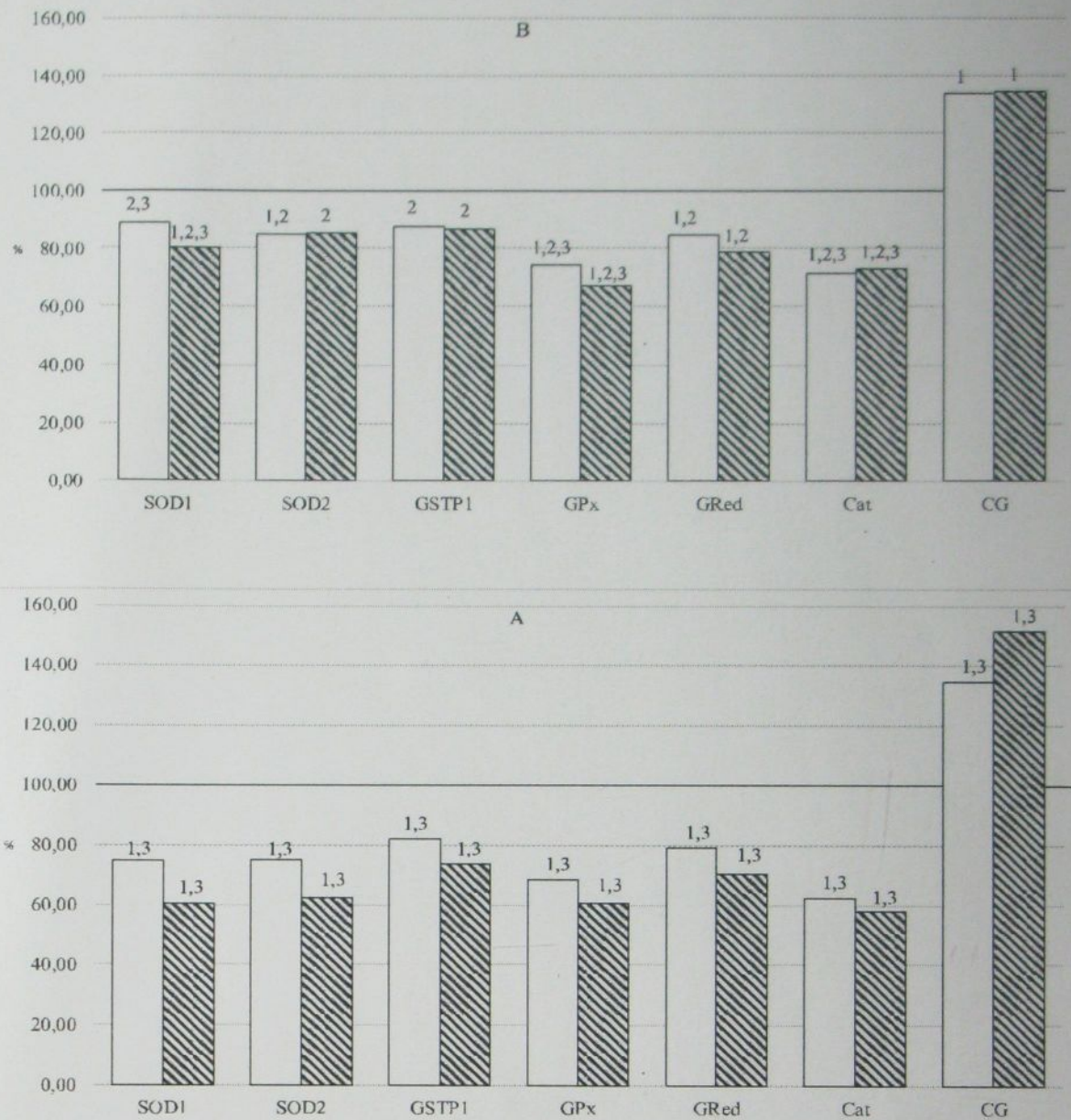


Figure 1. Activity of the AOS enzymes and the level of carbonyl groups (CG) in patients with different forms of lung tuberculosis before and after treatment (compared to the healthy individuals – 100%).

A – before treatment; B – after treatment

1 – significant differences between the groups of patients with tuberculosis and healthy individuals – ($p < 0,05$); 2 – significant differences in comparison with the baseline (before treatment) – ($p < 0,05$); 3 – significant differences between similar values in the A and B groups – ($p < 0,05$)

□ – infiltrative form; ▨ – disseminated form.

In the patients with infiltrative form of tuberculosis the change of the studied AOS enzymes correlated with a high level of carbonyl groups in the blood plasma (Table 3).

Table 3. Correlation between the level of carbonyl groups and the activity of the AOS enzymes in patients with infiltrative and disseminated form of tuberculosis

R-value (carbonyl groups/enzyme)	Tuberculosis patients (n=83)			
	Infiltrative form (n=42)		Disseminated form (n=41)	
	Before treatment	After treatment	Before treatment	After treatment
SOD1	-0,329*	0,080	-0,644*	-0,121
SOD2	-0,369*	-0,339*	-0,680*	-0,203
Cat	-0,807*	-0,477*	-0,858*	-0,085
GSTP1	-0,363*	-0,629*	-0,627*	-0,067
GRed	-0,763*	-0,481*	-0,717*	-0,266
GPx	-0,917*	-0,514*	-0,864*	-0,354*

* – $p < 0,05$

Discussion

The tuberculosis infection is characterized by severe inflammation. The oxidative stress plays a very important role in the pathogenesis of this specific inflammation due to the constant production of ROS and the depletion of non-enzymatic and enzymatic factors of the AOS [13]. The ROS are produced by neutrophils and macrophages [14] and destroy lung tissue cells in case of the AOS' failure, contributing to the spread of the inflammatory process.

It is known that in tuberculosis the activity of various AOS enzymes decreases, while the level of lipid peroxidation products gets higher [15]. *M. tuberculosis* is highly resistant to being killed due to the DNA damage, courtesy of the low H_2O_2 levels [16]. A similar situation is probable in the body of the patient. Our studies have shown a significant reduction in the activity of the first line group of the AOS enzymes that neutralize the ROS. The activity of the glutathione-dependent system enzymes (GPx, GRed and GSTP1) decreases as well. At the same time there is a strong positive correlation both within these enzyme systems and between them.

The study of the AOS enzyme activity in the peripheral blood allows a certain extent of the estimation of the total antioxidant status in patients with tuberculosis, given the severity of the tuberculosis process, the effect of anti-tuberculosis drugs and the nutritional antioxidants.

The simultaneous determination of the level of carbonyl groups and the AOS enzyme activity is important for assessing the antioxidant status [16]. It is known that the plasma level of carbonyl groups is associated with the increased production of the ROS, the reduction of the rate of their neutralization by the AOS enzymes and also the increased oxidation of proteins [17]. Our studies have shown a significant increase in the level of carbonyl groups in patients with tuberculosis, which points to its clear negative correlation with the activity of all the studied AOS enzymes.

It is possible that the increased level of carbonyl groups in plasma and the decreased AOS enzyme activity in patients with tuberculosis could be strongly associated with the oxidation of proteins, which include enzymes [18]. This might even be one of the mechanisms of the pathogenesis of the tuberculosis infection, which is known for the long, often chronic course.

Our studies have also shown that after two months of treatment the total antioxidant status in patients with tuberculosis improves. The activity of all the studied AOS enzymes increased while the level of carbonyl groups decreased. However, none of the values reached the level of healthy people. Along with this the degree of correlations changes significantly, which might be associated with the different reduction potential of each of these enzymes, the ambiguous effect of the anti-tuberculosis drugs on the ROS' production and the components of AOS, and also the different sensitivity of different protein molecules to the damaging effects of the drugs themselves.

Taking into consideration all said above, the patients with tuberculosis have got systemic impairments of the antioxidant status. The simultaneous study of the enzymatic activity of the various AOS components and the levels of carbonyl groups in the peripheral blood allows the determination of the extent of the oxidative stress and its changes in the course of the treatment. This will provide a more complete assessment of the effectiveness of the treatment with the inclusion of therapeutic measures aimed at restoring the balance in the production of the ROS and their neutralization via the components of the AOS to the standard treatment regimen.

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