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RESEARCH ARTICLE

Evaluation of oxidant and antioxidant system markers in patients with pulmonary tuberculosis before and after hospital treatment

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ABSTRACT

Introduction. Pulmonary tuberculosis remains a major cause of morbidity and mortality worldwide. According to data published by the World Health Organization in 2024, a total of 8.2 million people were newly diagnosed with TB in 2023, compared with 7.5 million in 2022, 7.1 million in 2019, and markedly higher than the 5.8 million and 6.4 million in 2020 and 2021, respectively.

Materials and methods. The prospective study involved 59 participants before and after treatment: 11 women (18.6%) and 48 men (81.4%). The participants were divided into 2 groups: group L_1 – patients with tuberculosis before treatment, and group L_2 – patients with tuberculosis after treatment. Serum levels of nitric oxide, malondialdehyde, glutathione reductase, and total antioxidant activity were measured using a spectrophotometric method.

Results. In the study, we demonstrated that nitric oxide and malondialdehyde serum concentrations were non-significantly higher in the L_2 group compared with the L_1 group. Glutathione reductase activity showed a significant decrease in antioxidant activity in the L_2 group, indicating reduced antioxidant capacity. Total antioxidant activity showed a non-significant decrease in the L_1 group compared with the L_2 group.

Conclusions. The results of the research demonstrated that the administered anti-tuberculosis treatment increased nitric oxide and malondialdehyde levels, and reduced glutathione reductase and total antioxidant activity. This phenomenon indicates the persistence of oxidative stress even after treatment. The levels of nitric oxide, malondialdehyde, glutathione reductase, and total antioxidant activity in patients with pulmonary tuberculosis may serve as biomarkers for monitoring disease progression.

Keywords: tuberculosis, oxidative stress, antioxidant, nitric oxide, malondialdehyde, glutathione reductase.

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Key messages

What is not yet known on the issue addressed in the submitted manuscript

The study demonstrated the involvement of pro- and antioxidant systems in the pathogenesis of pulmonary tuberculosis before and after hospital treatment, although this role is still not fully elucidated.

The research hypothesis

Pulmonary tuberculosis is associated with the amplification of oxidative stress and the reduction of antioxidant activity.

The novelty added by the manuscript to the already published scientific literature

For the first time, data will be obtained on laboratory manifestations of pro- and antioxidant activity in patients with pulmonary tuberculosis before and after hospital treatment. The study ad-

dressed an important issue, as applied research on the role of antioxidant status and oxidative stress markers in the course of tuberculosis, both before and after treatment, may prove useful in assessing disease progression and predicting future prognosis.

Introduction

Tuberculosis (TB) remains a major public health problem, with approximately 10 million new cases and 1.8 million deaths recorded each year worldwide.

According to data provided by the World Health Organization (WHO), the Republic of Moldova is among the 18 countries in the European region where tuberculosis control is a priority, as well as one of the 27 countries globally with a high incidence of multidrug-resistant tuberculosis (MDR-TB). According to WHO Pulmonary Tuberculosis Report 2024, 8.2 million people were newly diagnosed with TB in 2023, up from 7.5 million in 2022 and 7.1 million in 2019, and well above the 5.8 million and 6.4 million cases reported in 2020 and 2021, respectively [1]. These data indicate that TB prevention and control efforts worldwide remain insufficiently effective.

Mycobacterium tuberculosis can induce the production of reactive oxygen species (ROS) by activating both mononuclear and polymorphonuclear phagocytes, which possess antimicrobial activity. The increased level of free radical production, although intended to combat the pathogen, also has the potential to damage lung tissue. Normally, such tissue damage is limited by the host's enhanced antioxidant defense systems [1, 2]. However, weak antioxidant defenses have also been reported, which may expose the lung tissue of patients with pulmonary tuberculosis to oxidative damage [3, 4].

The oxidant-antioxidant balance is essential for maintaining lung function. Both an increase and a decrease in antioxidants can disrupt the physiological oxidant-antioxidant balance in favor of oxidants, leading to lung injury. Recent research suggests that oxygen and its related species (oxidants) may contribute to the pathogenesis of a considerable number of lung diseases. Increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) occurs in pulmonary tuberculosis and causes the phagocyte respiratory burst. Research results confirm that increased levels of circulating free radical activity are found in the pathogenesis of active pulmonary tuberculosis and, therefore, play an important role in the resulting fibrosis [5].

Mycobacterium tuberculosis is an intracellular pathogen that grows and replicates in host macrophages. It is well known that macrophages undergo a respiratory burst upon contact with this microorganism. These cells can generate huge amounts of reactive oxygen species (ROS), and ROS induce lipid peroxidation (LP), a chain reaction that affects unsaturated fatty acids located mainly in cell membranes, generating the end product malondialdehyde (MDA), which is responsible for some of the damaging effects of free radicals on DNA and cell membranes [6].

Lipid peroxidation products diffuse from the site of inflammation into the circulation and can be measured in blood samples. MDA is a three-carbon marker of oxidative stress–a low-molecular-weight aldehyde that can be produced from free radical attack on polyunsaturated fatty acids in biological membranes. The determination of MDA is used to monitor lipid peroxidation in biological samples. Although the concentrations of plasma antioxidant components can be measured individually, this process is time-consuming and involves additional costs. A more comprehensive assessment of the body's redox status can be achieved by measuring total antioxidant activity (TAA).

Recent studies further indicate that circulating markers of oxidative stress, such as nitric oxide (NO), are elevated in patients with pulmonary tuberculosis, whereas antioxidant parameters such as glutathione reductase (GR) activity are decreased. This imbalance between oxidants and antioxidants may contribute to the development of pulmonary function abnormalities and disease progression [7].

Materials and methods

The prospective observational study was conducted between 2019 and 2024 at the *Chiril Draganiuc* Institute of Pneumology in Chişinău and was approved by decision No. 31 of 18.05.2019, issued by the Research Ethics Committee of the *Nicolae Testemiţanu* State University of Medicine and Pharmacy.

A total of 59 patients with newly diagnosed pulmonary tuberculosis were enrolled. The study population included 11 women (18.6%) and 48 men (81.4%). The participants were divided into 2 groups: group L_1 – patients with TB before treatment, and group L_2 – patients with TB after inpatient treatment, according to individualized plans and assessed after one month of hospitalization. The levels of MDA, NO, TAA, and GR were measured in blood serum using a spectrophotometric method adapted for use with a Synergy H1 Hybrid Reader (BioTek Instruments, USA) and multimodal plates.

Nitric oxide dosage was performed according to the method described by Metelskaya V. and Gumanova N. [8]. The principle of the method involves deproteinization of the biological material, reduction of nitrates to nitrites, treatment of the supernatant with Griess reagent, and subsequent measurement of the optical density of the reaction product.

Determination of malondialdehyde, the final product of lipid peroxidation, was carried out according to the procedure described by Galaktionova L. and co-authors [9]. The method is based on the spectrophotometric identification of the colored trimethine complex formed by the interaction of thiobarbituric acid with MDA. The MDA concentration in

the analyzed sample is directly proportional to the color intensity, and the final result was expressed in μM/L in the studied biological serum.

Total antioxidant activity was assessed using the methodology described by Zhang M., adapted to the Synergy H1 Hybrid Reader multimodal plate assay (BioTek Instruments, USA) [10].

The activity of glutathione reductase (EC 1.6.4.2) was determined by the method described by Vlasova S. and co-authors [11], based on the Warburg optical test. The principle of the method is the measurement of NADPH consumption, used by GR to reduce GSSG, observed by the decrease in absorbance at 340 nm.

R Studio and Python programs were used for data analysis. For each parameter, the mean, standard deviation (SD), and the confidence interval (CI) were calculated. To compare the two groups, the non-parametric Mann-Whitney U test was applied, and the statistical significance of the differences was assessed using the p-value (considered significant at $p < 0.05$).

Sample and data collection procedure. All eligible study participants completed a questionnaire designed to obtain demographic information such as age and gender. A volume of 10 ml of venous blood was collected from the antecubital vein of the arm of each consenting participant. The test tubes for sample collection were labeled with a unique identification code for each participant. After 15 minutes–the time required for blood coagulation–the serum was immediately separated by centrifugation at 3000 rpm for 10 minutes. Subsequently, the obtained serum was transferred into sterile Eppendorf tubes. The serum samples were then preserved by freezing at a temperature of minus 40°C until the time of analysis.

Results

Based on demographic characteristics, a similar gender distribution in pulmonary tuberculosis before and after treatment was found in both groups, with a predominance of males – 48 participants (81.4%) compared to 11 females (18.6%), $p = 0.094$. This comparable distribution between the groups allowed for a relevant comparison.

The age difference between the two groups was found to be statistically significant (Wilcoxon rank-sum test = 2452.5, $p < 0.001$). In order to evaluate the practical significance of this finding, the rank biserial correlation coefficient was calculated, indicating a moderate effect size with a value of 0.41 (95% CI: 0.22–0.57). Therefore, the age difference, with higher values in the L_1 group, is not only statistically significant but also clinically relevant, potentially impacting the values of the analyzed indicators.

Serum malondialdehyde (MDA) concentrations were higher in the L_2 group (24.0 ± 8.3 μM/L) compared with the L_1 group, where values were 18.6 ± 5.2 μM/L, but the difference was not statistically significant ($p = 0.40$). Serum nitric oxide (NO) levels were significantly higher in the L_2 group, with a mean of 107.4 ± 99.7 μM/L versus 92.6 ± 43.5 μM/L ($p = 0.010$) in the L_1 group (Table 1).

Table 1. Pro-oxidant system indicators

Group	L_1n = 59	95% CI	L_2n = 59	95% CI	p
MDA (malondialdehyde), μM/L	18.6 ± 5.2	17–20	24.0 ± 8.3	22–26	= 0.40
NO (nitric oxide), μM/L	92.6 ± 43.5	81–104	107.4 ± 99.7	81–133	= 0.010*

Note: *The data presented include mean (standard deviation), 95% confidence interval, and statistically significant difference with the control group; L_1 – patients with TB before treatment; L_2 – patients with TB after inpatient treatment; CI – confidence interval; * – p<0.05; ** – p<0.01; *** – p<0.001; MDA – malondialdehyde; NO – nitric oxide.*

Serum total antioxidant activity (TAA) showed a slight, non-significant decrease in the L_2 group (3.6 ± 0.5 u/c) compared with the L_1 group (4.0 ± 1.0 u/c, $p > 0.90$). Conversely, serum glutathione reductase (GR) activity was significantly reduced in the L_2 group (298.2 ± 169.2 nM/s·L) compared with the L_1 group (704.6 ± 1,529.7 nM/s·L, $p < 0.001$) (Table 2).

Table 2. Antioxidant system indicators

Group	L_1n = 59	95% CI	L_2n = 59	95% CI	p
TAA (total antioxidant activity), u/c	4.0 ± 1.0	3.7–4.3	3.6 ± 0.5	3.7–4.0	>0.9
GR (glutathione reductase) nM/s·L	704.6 ± 1,529.7	306–1,103	298.2 ± 169.2	254–342	<0.001*

Note: *The data presented include mean (standard deviation), 95% confidence interval, and statistically significant difference with the control group; L_1 – patients with TB before treatment; L_2 – patients with TB after inpatient treatment; CI - confidence interval; * – p<0.05; ** – p<0.01; *** – p<0.001; TAA – total antioxidant activity; GR – glutathione reductase.*

Discussion

The study analyzed oxidative and antioxidative parameters in patients diagnosed with pulmonary tuberculosis before and after hospital treatment. Oxidative stress occurs as a result of increased production of reactive oxygen species during the respiratory burst, concomitantly with a decrease in the effects of antioxidants [12, 13]. This imbalance disrupts normal lung function and host immune responses, contributing to the development of the disease through the body's inability to effectively eliminate oxidative stress and causing pulmonary dysfunction [13, 14]. Elevated MDA and NO values in the serum of patients after anti-tuberculosis treatment indicate a high level of lipid peroxidation and an imbalance of redox homeostasis [15]. According to data from the specialized literature, the high levels of MDA and NO persist even after the end of treatment. Circulating antioxidants, such as total TAA and GR mentioned in this study, appear to be insufficient to counterbalance oxidative stress in pulmonary tuberculosis, and the accumulation of free radicals in lung tissue leads to the attack of cell membrane lipids and lipid peroxidation [13, 16]. Thus, the existence of a deficiency in the antioxidant system at the alveolar level in pulmonary tuberculosis is confirmed. A defining feature of the evolution of this condition is the association between hypoxia, oxidative stress, and inflammation.

Regarding the distribution of patients by gender and age, there was a predominance of men compared with women. In both studied groups, the predominance of patients aged between 18 and 55 created optimal conditions for comparability of the results obtained.

The results of the study highlight the important role of glutathione reductase in both innate and cellular immunity against tuberculosis infection. Oxidative stress related to inflammation has been implicated in the pathogenesis of fibrosis and lung dysfunction in patients with pulmonary tuberculosis [17,18]. The specialized literature reports that several circulating markers of free radical activity are elevated in patients with pulmonary tuberculosis, and some of these markers remain elevated even after the completion of anti-tuberculosis therapy, indicating ongoing oxidative stress that may contribute to the decrease in GR levels [19].

Finally, nutritional status directly influences the health and proper functioning of all body systems, including the immune system, which plays an essential role in protecting against infectious diseases, such as pulmonary tuberculosis. Considering that cellular immunity is the body's main line of defense against tuberculosis, malnutrition becomes a significant risk factor for the onset and progression of this disease.

Conclusions

As a result of the analysis of oxidative system biomarkers, such as NO and MDA, it was found that after the administration of anti-tuberculosis treatment, the levels of NO and MDA increased, while the values of TAA and GR decreased, indicating the persistence of oxidative stress even after treatment. These biomarkers provide important information both before and after the initiation of standard therapy. They are necessary for evaluating treatment efficacy, as well as for the development of new therapeutic strategies that include antioxidants, and can also be used to monitor the evolution of the disease and lung lesions.

The presence of elevated oxidative biomarkers and decreased antioxidant parameters after anti-tuberculosis treatment indicates that oxidative stress remains active in the body, which may negatively influence pulmonary recovery. Monitoring these markers may be essential for optimizing therapy and may pave the way for the integration of antioxidants into tuberculosis treatment strategies in the future.

Competing interests

None declared.

Authors' contributions

All the authors participated in the study design and contributed to drafting the manuscript. The authors critically reviewed the work and approved the final version of the manuscript.

Ethics approval

The research project was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy (Minutes No. 31 from 18.05.2019).

Patient consent

Obtained.

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