Thiol – Disulphide Homeostasis as a Novel Oxidative Stress Marker in Lung Tuberculosis Patient

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Abstract

ABSTRACT Aims of Study: To compare dynamic thiol/disulfide homeostasis between patients with with lung tuberculosis and healthy controls. Methods: Our study included 50 patients with active lung tuberculosis and 50 healthy controls. Serum thiol/disulfide was measured with a new automated spectrometric method developed by Erel and Neselioglu, and results were compared statistically. Results: We found that native and total thiol levels were significantly decreased in patients with lung tuberculosis, disulfide/native thiol and disulfide/total thiol levels were found to be higher in lung tuberculosis patients when compared with the control group. However, disulfide levels were higher in the control group than in the patient group. Conclusions: Based on the results of this study, it can be said that oxidative stress is closely associated with lung tuberculosis pathogenesis. There is a need for new studies that will show the possible effects of oxidative stress on lung tuberculosis pathogenesis.

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Results: We found that native and total thiol levels were significantly decreased in patients with lung tuberculosis, disulfide/native thiol and disulfide/total thiol levels were found to be higher in lung tuberculosis patients when compared with the control group. However, disulfide levels were higher in the control group than in the patient group.

Conclusions: Based on the results of this study, it can be said that oxidative stress is closely associated with lung tuberculosis pathogenesis. There is a need for new studies that will show the possible effects of oxidative stress on lung tuberculosis pathogenesis.

KEY WORDS

Tuberculosis, Oxidative Stres, Thiol – Disulphide Homeostasis.

WHAT'S KNOWN

Tuberculosis is major public health problem worldwide. Until recent years, it remains as one of the most important causes of death. Therefore, biomarkers are very important for effective and accurate determinations in the diagnosis, treatment monitoring and result of tuberculosis disease. The currently available diagnostic technology for tuberculosis detection is inadequate.

WHAT'S NEW

Novel biomarkers are needed in the prognosis and treatment process of the disease. There has been no report about thiol levels in lung tuberculosis patients, so far. To our knowledge, this study is the first study that investigates thiols and thiol/disulphide homeostasis in patients with lung tuberculosis. For this purpose, determination of dynamic thiol/disulphide status in diseases where oxidative stress plays a major role in pathogenesis would be important.

INTRODUCTION

Tuberculosis (TB) is a chronic, necrotic infection created by a group of mycobacteria, defined primarily as Mycobacterium tuberculosis (MTB) complex in all organs of the body, primarily the lung [1]. TB is a communicable disease that is a major cause of ill health, one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV/AIDS) according to World Health Organization (WHO) report in 2019 [2]. Lung tuberculosis is tuberculosis which attacks the lung tissue. Transmission generally occurs from an infected person to another person via droplets or with coughing up blood or contaminated sputum [3].

Most of the radicals formed in the human body are derived from oxygen [4]. The formation of highly reactive oxygen containing molecular species is a normal consequence of a variety of essential biochemical reactions. In healthy conditions at the cellular level, there is a critical balance exists between the free radical generation and the antioxidant defense [5]. Oxidative stress is excessive exposure to oxidant and / or decreased antioxidant capacity. Free radicals react with many organic and inorganic compounds, including polyunsaturated fatty acids of DNA, protein and cell phospholipids. Free radicals play a role in the pathogenesis of many diseases such as atherosclerosis, neurodegenerative diseases, cancer, allergies, diabetes, cataracts [6, 7].

Lung is the organ most affected by oxidants because it is under the influence of air pollution and blood-borne oxidants. It is also the organ that meets the most oxygen. It was observed that oxidative stress was increased in tuberculosis, and it was stated in the researches that the incidence of lung cancer was higher, especially in patients with chronic lung tuberculosis [8].

The oxidative environment normally helps to kill pathogenic microorganisms. However, in the intracellular pathogen of MTB, the opposite can grow well in macrophages in environments with high oxygen concentrations [9]. Macrophages undergo respiratory burst upon contact with this microorganism. These cells are capable of producing large amounts of reactive oxygen species (ROS) [10]. MTB infection can induce oxidative stres [11].

Recent studies have revealed significant correlations between oxidative stress (OS) and certain diseases. There are various biochemical markers used with the aim of identifying OS and inflammation. One of these markers is dynamic thiol/disulphide balance. Thiol / disulphite homeostasis (TDH) plays a critical role in many cellular activities such as antioxidant protection, detoxification, cell growth, aopoptosis, signal transduction, and enzyme activities [12, 13]. Thiols, forming a significant proportion of total antioxidants in the body. They are compositions containing sulfur and play a substantial role in aiding the body's defense versus reactive oxygen species. Plasma thiols scavenge free radicals through a variety of mechanisms. They are commonly accepted as playing a physiologic role by acting as antioxidants [14].

There is increasing evidence showing that abnormal thiol/disulphide homeostasis situations play a role in pathogenesis of a variety of diseases such as diabetes, cancer, cardiovascular disease, chronic kidney disease, liver disorder and autoimmune subclinical hypothyroidism (Hashimoto thyroiditis) [15-20].

METHODS

The goal of this study was to evaluate thiol/disulphide balance as a novel marker of OS in TB patients and to investigate changes occurring in the oxidant antioxidant system, TDH parameters (serum native thiol, total thiol and disulphide levels and disulphide/native thiol, disulphide/total thiol, native thiol/total thiol ratios) measurements in TB.

This study was performed with respect to the recommendations put forward via the Declaration of Helsinki. The study protocol was approved by the Ethical Committee and each participant gave written, informed consent. 50 patients with active lung tuberculosis referred to the Chest Diseases department of Harran University Medical Faculty Hospital (29 male, 21 female) and 50 healthy controls (29 male, 21 female) were included in the study.

Venous blood samples from the patients and healthy controls in the study were collected. Plasma blood samples were centrifuged at 1500 rpm for 10 min and serum was obtained. The separated serum was immediately placed in Eppendorf tubes and these samples were stored at -80°C until used.

Thiol/disulphide homeostasis evaluation had performed by a fully-automatic method, developed by Erel and Neselioglu [12]. Disulphide bonds are first reduced with sodium borohydride to create functional thiol groups. Unused reducing agent, sodium borohydride, was removed with formaldehyde to prevent reduction of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). All thiol groups, including reduced and native thiol groups, were later fixed by reactions with DTNB. Half of the difference between total thiol and native thiol determined the dynamic disulphide amount. After determining native and total thiols, disulphide levels, disulphide/total thiol, disulphide/native thiol, and native thiol/total thiol ratios were calculated.

Statistical Analysis

Statistical analysis was completed using IBM SPSS 23.0 (SPSS for Windows, SPSS Inc., Chicago, IL, USA). The Shapiro–Wilks test was used for normality testing of native thiol, total thiol, disulphide level, disulphide/native thiol, disulphide/total thiol, and native thiol/total thiol ratios. The groups displayed normal distribution. The independent samples t-test, among parametric tests, was used to investigate whether there were considerable differences between the groups. $P < 0.05$ was accepted as statistically significant.

RESULTS

50 patients with lung tuberculosis and 50 healthy volunteers were included in the study. 29 patients with lung tuberculosis (58%) were male and 21 patients with lung tuberculosis (42%) were female. 29 control group (58%) were male and 21 control group (42%) were male. The mean age of the patient group diagnosed

with lung tuberculosis was 33.84 +- 12.40 years, and the mean age of the control group was 34.76 +- 12.17 years. There was no significant difference between the groups in terms of age. There was no significant difference between the groups in terms of gender.

The evaluation of TB and control group in terms of thiol and disulphide levels are shown in table 1.

	Groups	**N**	**Average ± SD**	**P**
Native thiol (SH) (μμολ/Λ)	Control	50	388,22± 44,33	0,000
	Patient (TB)	50	252,52± 61,28	
Total thiol (TT) (μμολ/Λ)	Control	50	419,22± 47,08	0,000
	Patient (TB)	50	277,18± 64,83	
Disulphide(SS) (μμολ/Λ)	Control	50	15,50± 6,39	0,020
	Patient (TB)	50	12,33± 6,97	
%Disulphide/Native thiol (SS/SH)	Control	50	4,035± 1,77	0,034
	Patient (TB)	50	5,10± 3,00	
%Disulphide/Total thiol (SS/TT)	Control	50	3,69±1,48	0,044
	Patient (TB)	50	4,50± 2,40	
%Native thiol/Total thiol (SH/TT)	Control	50	92,63± 2,96	0,043
	Patient (TB)	50	90,10± 4,79	

Table 1 Evaluation of TB and control group in terms of thiol and disulphide levels.

Native thiol measurements were 252.52 ± 61.28 in the TB group and 388.22 ± 44.33 in the control group. There was a statistically significant difference between the groups in terms of native thiol levels, the mean native thiol level ($p < 0.01$) is lower in the TB group when compared to the control group (Figure 1).

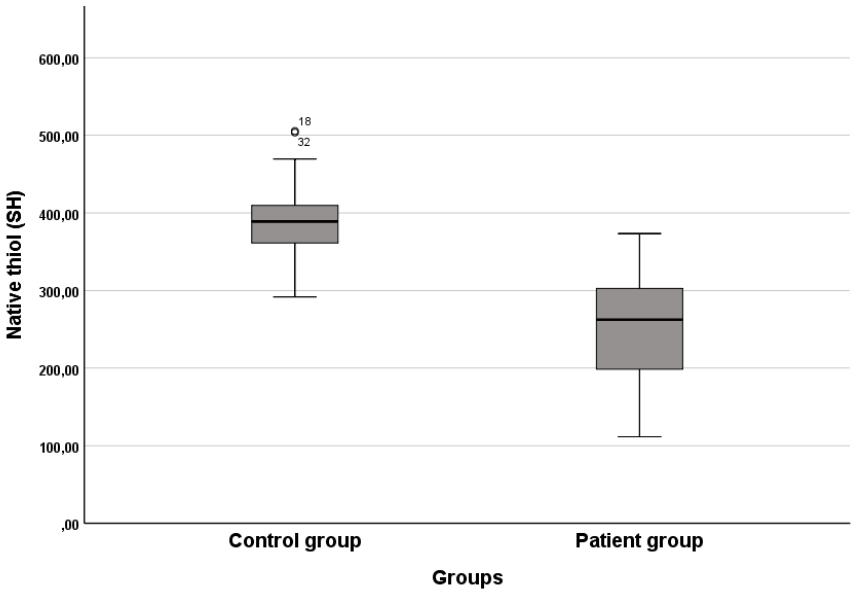

Figure 1 Native thiol levels between groups.

There was a statistically significant difference between the groups in terms of total thiol levels and TB group measurements were found to be lower than the control group ([277.18 ± 64.83] vs [419.22 ± 47.08], p <0.01), (Figure 2).

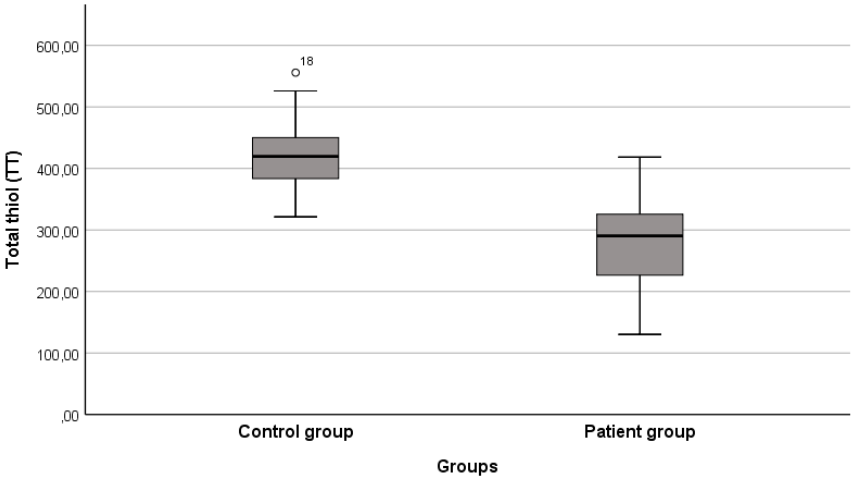

Figure 2 Total thiol levels between groups.

Disulphide level is higher in the TB group when compared to the control group and this difference was statistically significant ([12.33 ± 6.97], [15.50 ± 6.39], p <0.05), (Figure 3).

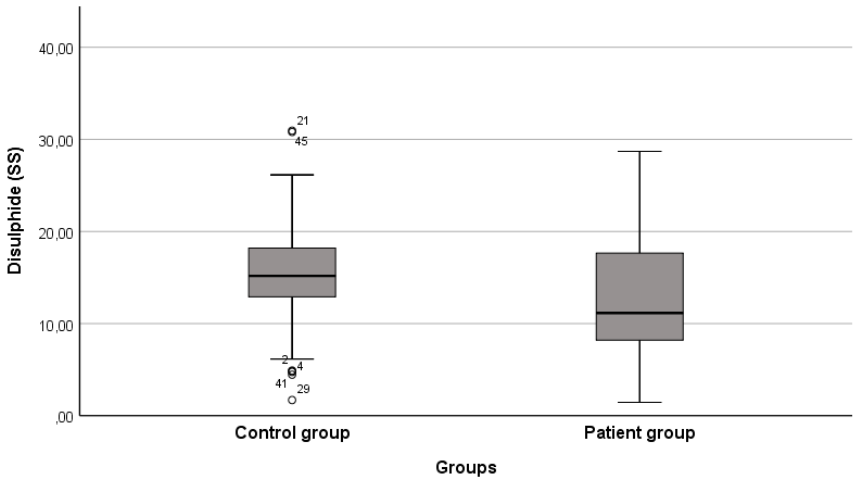

Figure 3 Disulphide levels between groups.

Disulphide/Total thiol ratio was higher in the TB group than the control group, and this difference was statistically significant ([4,50± 2,40] vs [3,69±1,48], p <0.05), (Figure 4).

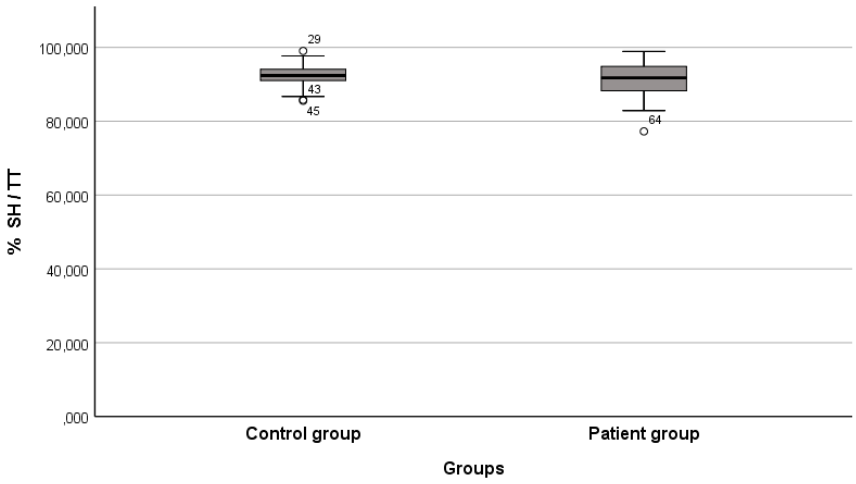

Figure 4 Disulphide/Total thiol ratio between groups.

The disulphide/native thiol ratio was higher in the TB group than the control group, and this difference was statistically significant ([5,10± 3,00], [4,035± 1,77], p <0.05), (Figure 5).

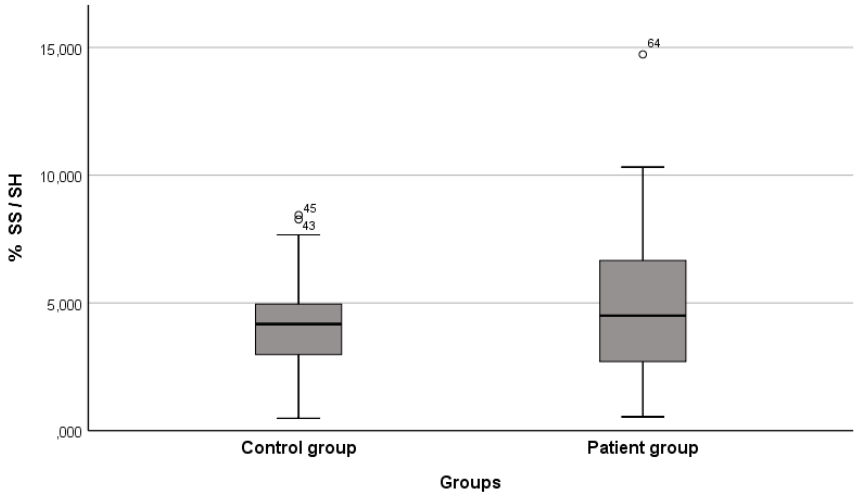

Figure 5 Disulphide/Native thiol ratio between groups.

A statistically significant difference was determined between the groups in native thiol/total thiol ratios,). The Native thiol/Total thiol ratio was lower in the PTE group than the control group ([90,10± 4,79], [92,63± 2,96], p <0.01), (Figure 6).

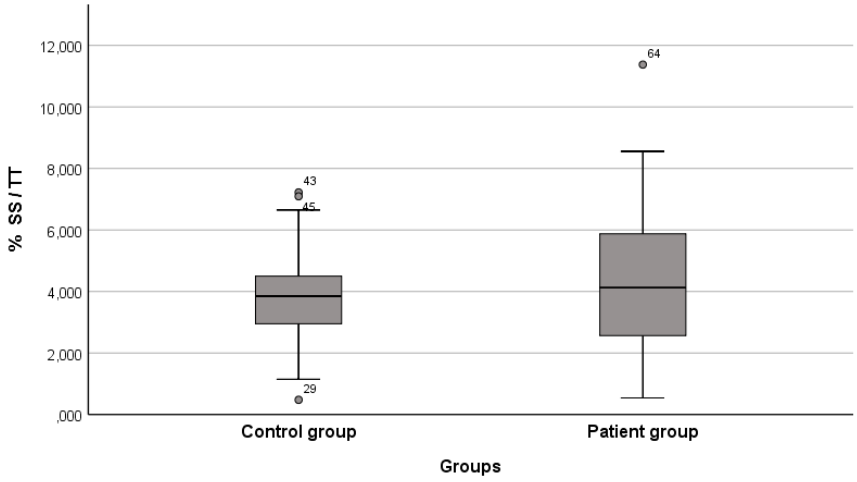

Figure 6. Native thiol /Total thiol ratio between groups.

DISCUSSION

Tuberculosis is major public health problem worldwide [21]. It is estimated that approximately one-third of the world's population is living with latent TB. Until recent years, it remains as one of the most important causes of death. Therefore, biomarkers are very important for effective and accurate determinations in the diagnosis, treatment monitoring and result of tuberculosis disease. The currently available diagnostic technology for TB detection is inadequate. Novel biomarkers are needed in the prognosis and treatment process of the disease [22].

Free radical production has been described in different cancers, lung injury and pulmonary emphysema

[23-26]. Also degenerative lung diseases such as tuberculosis are associated with lung oxidant-antioxidant imbalance [27]. Mycobacterium tuberculosis is intracellular pathogens, which grow and replicate in the host macrophages. It is well known that macrophages undergo respiratory burst after contact with this microorganism. Increased amounts of ROS are produced as a result of respiratory burst. ROS released from macrophages cause tissue damage in respiratory tract infections [23]. Containing sulfurous amino acid molecules are more sensitive to free radicals. The sulphurous amino acids cysteine and cystine are also sensitive to free radical attack. Proteins with a large number of disulfide bonds such as IgG and albumin break down their three-dimensional structure. Thus, they can not perform their normal functions. Changes in protein structure can lead to changes in antigenicity and proteolysis. Radicals can react with membrane proteins and cause impairment of the functions of enzyme, neurotransmitter and receptor proteins [28, 29].

The plasma thiol pool mainly comprises albumin thiols and protein thiols, smaller amounts of low-molecular-weight thiols such as cysteine (Cys), cysteinyl glycine, homocysteine, glutathione, and γ-glutamyl Cys [12]. Measuring plasma total thiol levels and defining thiol / disulfide homeostasis is a good indicator of excessive free radical formation in many diseases. it also provides important information about the extent of free radical-mediated oxidation that causes protein damage [14, 30].

Erel and Neselioğlu reported higher plasma disulfide levels in degenerative diseases such as obesity, pneumonia, bronchiolitis and diabetes mellitus than healthy groups; In another word, thiol / disulfide homeostasis has shifted towards disulfide [12]. Topuz et al. detected that serum thiol levels in acute pulmonary thromboembolism (APE) patients were significantly low compared to a control group and disulfide level and disulfide/total thiol ratio were higher in APE group than control group [31]. Recent studies have shown that the thiol/disulphide homeostasis is disturbed in lung diseases such as infectious pneumonia [32], obstructive sleep apnea syndrome [33], COPD, asthma [34] and silicasis [35]. Plasma total thiol levels are reduced in patients with COPD or asthma [36]. Solak et al have found NT and TT levels significantly lower in smokers in comparison with the control group [37]. In a previous study, serum SOD activities were significantly decreased in tuberculosis patients compared to healthy controls, and serum MDA levels were increased [38, 39]. This result indicates that oxidative stress is increased in patients with lung tuberculosis. Durak et al. investigated pleural fluid and serum superoxide dismutase (SOD) values in patients with lung cancer, tuberculosis and heart failure, and found that pleural fluid and serum SOD values of all patient groups, being the highest in the tuberculosis group, were higher than the control group values. As a result, they stated that this enzyme activity could be used as a nonspecific prognostic indicator in detecting cellular and mitochondrial tissue damage [24].

Limited data is available on the relationshipbetween thiol/disulphide homeostasis and lung diseases. Determination of dynamic thiol/disulphide status in diseases where oxidative stress plays a major role in pathogenesis would be important. There has been no report about thiol levels in lung tuberculosis patients, so far. To our knowledge, this study is the first study that investigates thiols and thiol/disulphide homeostasis in patients with lung tuberculosis. In this study, we found that native and total thiol levels were significantly decreased in patients with lung tuberculosis. We think that the ROS released from the increased macrophages in lung tuberculosis patients decrease the thiol levels by oxidizing the thiols. In this study, disulfide/native thiol and disulfide/total thiol levels were found to be higher in lung tuberculosis patients when compared with the control group. However, disulfide levels were higher in the control group than in the patient group.

CONCLUSION

According to the results of our study, it can be clearly said that thiol / disulphide homeostasis is affected in lung tuberculosis. This may play a role in the pathogenesis of this disease, and measuring this parameters can provide an overview for understanding the disease process. To our knowledge, our study is the first to evaluate the dynamic thiol–disulphide homeostasis in the serum of patients with lung tuberculosis. The results of our study showed that this newly developed test can be used as an assayed, accurate and novel oxidative stress marker in the pathogenesis of lung tuberculosis. Evaluation of thiol/disulfide parameters in lung tuberculosis patients can make a significant contribution to assessing and monitoring patients with lung tuberculosis patients. There is a need for new studies that will show the possible effects of oxidative

stress on lung tuberculosis pathogenesis.

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Author contribution

All authors have participated in article preparation.

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