Thiol – disulphide homeostasis as a novel oxidative stress marker in pulmonary thromboembolism

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Abstract

Aim of Study: Our aim is to compare dynamic thiol/disulphide homeostasis between patients with acute pulmonary thromboembolism (PTE) and healthy controls. Methods: Our study included 45 patients who were diagnosed with acute PTE and 50 healthy controls. Serum thiol/disulphide was measured. Results: We found that the native thiol, total thiol, native thiol/total thiol levels were significantly lower in the patient group than the control group, while the disulphide, disulphide/natural thiol, disulphide/total thiol levels were significantly higher. Conclusion: We have seen that in patients diagnosed with acute PTE, the oxidant-antioxidant balance shifts towards the oxidative direction. The disulfide/natural thiol ratio can be considered as an oxidative stress parameter in acute PTE. We think that the deterioration in thiol disulfide balance, together with clinical, laboratory and radiological findings, may have diagnostic value in acute PTE patients.

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Aim of Study: Our aim is to compare dynamic thiol/disulphide homeostasis between patients with acute pulmonary thromboembolism (PTE) and healthy controls.

Methods: Our study included 45 patients who were diagnosed with acute PTE and 50 healthy controls. Serum thiol/disulphide was measured.

Results: We found that the native thiol, total thiol, native thiol/total thiol levels were significantly lower in the patient group than the control group, while the disulphide, disulphide/natural thiol, disulphide/total thiol levels were significantly higher.

Conclusion: We have seen that in patients diagnosed with acute PTE, the oxidant-antioxidant balance shifts towards the oxidative direction. The disulfide/natural thiol ratio can be considered as an oxidative stress parameter in acute PTE. We think that the deterioration in thiol disulfide balance, together with clinical, laboratory and radiological findings, may have diagnostic value in acute PTE patients.

KEYWORDS: Acute Pulmonary thromboembolism, oxidative stress, thiol–disulphide homeostasis.

WHAT'S KNOWN

Pulmonary thromboembolism is one of the leading causes of death due to its high morbidity and mortality. When early diagnosis and treatment is made, there is a serious decrease in mortality rates. Therefore, biomarkers are very important for effective and accurate determinations in the diagnosis, treatment monitoring and result of PTE disease. The currently available diagnostic technology for PTE detection is not specific.

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 pulmonary thromboembolism patients, so far. To our knowledge, this study is the first study that investigates thiols and thiol/disulphide homeostasis in patients with pulmonary thromboembolism (with subgroups). For this purpose, determination of dynamic thiol/disulphide status in diseases where oxidative stress plays a major role in pathogenesis would be important.

INTRODUCTION

Pulmonary thromboembolism (PTE) occurs when a blood clot originating from any systemic vein blocks one or more branches of the pulmonary arteries [1]. PTE can develop as a result of an acute or chronic process. PTE; It is divided into three types: massive with high mortality, submassive with moderate mortality, and non-massive with low mortality [2]. PTE; It is the third most common cardiovascular disease after acute myocardial infarction and stroke [3]. Some patients die before being diagnosed. Therefore, its net prevalence is not known [4]. Clinical symptoms and signs; It may vary depending on the size, number, location of the embolus, the development of an infarction, the resolution rate, the recurrence or not, the age of the patient and the reserve of cardiopulmonary functions. The most common symptoms; dyspnea, pleuritic chest pain, cough, syncope, and hemoptysis [5]. Suspecting pulmonary thromboembolism is the most important step in diagnosis. Suspicion of disease should be based on risk factors, symptoms, examination findings, electrocardiogram, biochemical data, and chest radiography findings. clinical evaluation alone is not reliable to diagnose or rule out PTE. However, both clinical follow-up and clinical predictive rules are useful in determining the probability of PTE before examination [6].

Thiol; It is an organic compound containing sulfhydryl (-SH) group, which has a critical role in preventing the formation of oxidative stress in cells. Plasma thiols show pro-oxidant or mostly antioxidant effects in physiological and biological events. Thiol groups of sulfur-containing amino acids (such as cysteine, methionine) in protein are the primary target of reactive oxygen species (ROS). ROS transfer their electrons to other species. Thiols have standard reducing potential and therefore act as fast electron acceptors. In this way, the oxidant is reduced by thiols and neutralized to a less harmful product. The thiol molecule is

oxidized and transformed into disulphide (C-S-S-C). This reaction is reversible and normally exists in the body in equilibrium. [7].

In previous studies, native thiol and total thiol values were found to be lower in the patient group compared to the control group in cases such as pneumonia and stomach cancer [8, 9], and these values were found to be higher in psoriasis and lichen planus patients [10, 11]. Disulphide values were found to be lower in colon cancer, multiple myeloma and fibromyalgia compared to the control group [9, 12], whereas these values were found to be higher in diabetes and pneumonia [13, 7].

It is known that oxidative stress plays an important role in the pathogenesis of cell and tissue damage. Antioxidants are thought to be an effective treatment method for preventing oxidative tissue damage [7]. There are various biochemical markers used with the aim of identifying oxidative stress (OS) and inflammation. One of these markers is dynamic thiol/disulphide balance. Thiol / disulphide homeostasis (TDH) plays a critical role in many cellular activitie, such as antioxidant protection, detoxification, cell growth, apoptosis, signal transduction, and enzyme activities [7, 14]. 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 [15]. The aim of this study is to determine how thiol disulphide balance changes in PTE cases and to examine its usability as a new biomarker.

METHODS

We performed this study regarding the recommendations put forward via the Declaration of Helsinki. The study protocol was approved by the Harran University Faculty of Medicine Ethics Committee and each participant gave written, informed consent. 95 patients, including 45 patients over the age of 18 who were diagnosed with acute PTE (non-massive and submassive), and 50 healthy volunteers over the age of 18 with a normal physical examination, who applied to the Harran University Faculty of Medicine Chest Diseases Polyclinic in 2019, were included in our study.

We followed the following exclusion criteria in these cases. Patients with neurological diseases, patients with diabetes, patients with cardiovascular disease, patients with cancer, patients with rheumatoid arthritis, patients with kidney disease, individuals with a history of drug, smoking, alcohol use for any reason within the last 7 days, patients with infectious diseases, other respiratory diseases, those with hypoxia, anoxia, metabolic and different systemic diseases were excluded.

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

Thiol/disulphide homeostasis evaluation had performed by a fully automatic method, developed by Erel and Neselioglu [7]. 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 nitro benzoic 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

These cases were examined in terms of age, gender, native thiol, total thiol, disulphide, disulphide/native thiol, disulphide/total thiol, native thiol/total thiol levels. Statistical analysis was completed using IBM SPSS 25.0 (SPSS for Windows, SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk 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. Independet simple T test was used in our data suitable for normal distribution, and

Mann Whitney U test was used in our non-normally distributed data. Crosstabs and frequency tests were used for demographic data and frequency analysis of variables P <.05 was accepted as statistically significant.

RESULTS

We included 45 patients with acute pulmonary embolism and 50 healthy volunteers in the study. 23 of our patients were non-massive and 22 of them were submassive patients. 25 patients with PTE (55.6%) were female and 20 patients with PTE (44.4%) were male. 26 control group (52%) were female and 24 control group (48%) were male. We showed the distribution of the patient and control groups by gender in table 1. There was no significant difference between the groups in terms of gender (p = 0.73).

The mean age of the patient group diagnosed with acute pulmonary embolism was 56.87 ± 16.30 years, and the mean age of the control group was 52.84 ± 14.23 years. There was no significant difference between the groups in terms of age (p = 0.20)

Serum native thiol, total thiol and disulphide levels and disulphide/natural thiol,

disulphide/total thiol, natural thiol/total thiol ratio measurements in PTE and control group were shown in table 2. Native thiol measurements were 228.65 ± 47.96 in the PTE group and 301.62 ± 58.34 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 =0.000) is lower in the PTE group when compared to the control group. When we evaluated the subgroups of our patients, native thiol levels in non-massive and submassive patient groups were significantly lower than the control group (260.12 ± 40.82, p =0.003, 195.75 ± 29.27, p =0.001). Native thiol levels in non-massive patient group was significantly higher than the submassive patient group (p =0.000).

There was a statistically significant difference between the groups in terms of total thiol levels, and PTE group measurements were found to be lower than the control group (271.73 ± 48.33, 329.76 ± 61.65, p =0.000). While there was no significant difference in total thiol levels between the non-massive patient group and the control group (299.95 ± 42.70, p =0.078), the total thiol levels of the sub-massive patient group was significantly lower than the control group (242.23 ± 34.64, p =0.000). Total thiol levels in non-massive patient group was significantly higher than the submassive patient group (p =0.001).

Disulphide level is higher in the PTE group when compared to the control group, and this difference was statistically significant (21.54 ± 5.19, 14.07 ± 4.04, p =0.000). Disulphide levels in non-massive and submassive patient groups were significantly higher than the control group (19.91 ± 4.80, p =0.000, 23.24 ± 5.15, p =0.000). There was no significant difference in disulphide levels between the non-massive and submassive patient group (p =0.055).

The disulphide/native thiol ratio was higher in the PTE group than the control group, and this difference was statistically significant (9.84 ± 3.13, 4.81 ± 1.61, p =0.000). Disulphide/native thiol ratio in non-massive and submassive patient groups were significantly higher than the control group (7.81 ± 2.33, p =0.000, 11.96 ± 2.40, p =0.000). Disulphide/native thiol ratio in non-massive patient group was significantly lower than the submassive patient group (p =0.000).

Disulphide/total thiol ratio was higher in the PTE group than the control group, and this difference was statistically significant (8.11 ± 2.17, 4.35 ± 1.32, p =0.000). Disulphide/total thiol ratio in non-massive and submassive patient groups were significantly higher than the control group (6.69 ± 1.69, p =0.000, 9.59 ± 1.56, p =0.000). Disulphide/total thiol ratio in non-massive patient group was significantly lower than the submassive patient group (p =0.000).

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 (83.77 ± 4.35, 91.29 ± 2.64, p =0.000). Native thiol/total thiol ratio in non-massive and submassive patient groups were significantly lower than the control group (86.61 ± 3.38, p =0.000, 80.81 ± 3.11, p =0.000). Native thiol/total thiol ratio in non-massive patient group was significantly higher than the submassive patient group (p =0.000).

DISCUSSION

PTE is one of the leading causes of death because of its high morbidity and mortality. If untreated, the mortality rate of up to 30% can be reduced to 3-8% as because of early diagnosis and treatment. Despite technological advances in diagnosis, its symptoms, radiological and laboratory findings are not specific [16, 17]. The incidence of pulmonary embolism increases with age [5]. In our study, we found the mean age of the patient group to be 56.87 ± 16.30 years.

Thiols are sulfur analogs of alcohols, which are formed by bonding a sulfur and hydrogen atom to the carbon atom, containing sulfhydryl (-SH) groups. Albumin and other proteins make up the most of the plasma thiol pool, while the remaining small part is low molecular weight thiols such as cysteine, cysteinyl, glycine, glutathione, homocysteine and gamma-glutamylcysteine. Disulphide (RS-SR) bonds are formed when thiols (R-SH) undergo an oxidation reaction by various oxidants. The disulphide bonds formed can be reduced back to the thiol groups, thus maintaining the dynamic thiol / disulphide balance [7]. There are some studies that show that dynamic thiol disulphide balance is affected in many diseases [8-11, 18]. In the study of Parlak et al. investigating the relationship between thiol/disulphide balance status and HDL cholesterol level with pulmonary embolism, it was reported that native thiol, total thiol and HDL-C values were significantly lower in the patient group compared to the control group. It was reported that % disulphide/native thiol was significantly lower in the control group compared to the patient group. It was reported that there was no significant difference in disulphide level between the patient and the control group [19]. Topuz et al. In the study investigating the prognostic significance of thiol disulphide homeostasis in patients with acute pulmonary thromboembolism, it was found that the mean native thiol level was lower in the pulmonary thromboembolism group, the disulphide level and the % disulphide/total thiol ratio was higher than the control group. Among the limitations of this study, it was stated that the patients had some comorbidities such as diabetes mellitus and atherosclerosis, which can change the thiol disulphide balance [20].

There are a few studies investigating thiol/disulphide balance in PTE patients. Determination of dynamic thiol/disulphide status in diseases where oxidative stress plays a major role in pathogenesis would be important. In our study, we found that native thiol, total thiol, native thiol/total thiol levels in the patient group were significantly lower than the control group. We found that the disulphide, disulphide/native thiol, disulphide/total thiol values were significantly higher in the patient group compared to the control group. The result of native thiol, total thiol and disulphide/native thiol in our study is in parallel with the result of both Parlak et al. and Topuz et al. study. However, although Parlak et al. did not find a significant difference in the level of disulphide between the groups, we found significantly higher disulphide levels in the patient group in our study. In addition, although some comorbidities that would change the thiol disulphide balance were not excluded in the study conducted by Topuz et al., it excluded them from our study. The results we got in our study show that there is a significant difference not only between the patient and control groups, but also between the subgroups of the patients.

CONCLUSION

In our study, we found a significant difference between both patient control and subgroups. However, massive PTE patients were not included in our study because there were no massive PTE patients who met the exclusion criteria in our hospital during our study. In this study, we observed that the oxidant-antioxidant balance shifted to the oxidative direction in patient groups diagnosed with acute PTE. We thought that the oxidizing disulfide/natural thiol ratio could be considered as an oxidative stress parameter in acute PTE. We think that the deterioration in thiol disulfide balance together with clinical, laboratory and radiological findings may have diagnostic value in patients with acute PTE. However, since studies on the subject are not yet at a sufficient level, more comprehensive studies that include all subgroups are needed.

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Author contribution

All authors have participated in article preparation.

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