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THIOL – DISULPHIDE HOMEOSTASIS AS A NOVEL OXIDATIVE STRESS MARKER IN PULMONARY THROMBOEMBOLISM

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ABSTRACT

Aim: Our aim of this study is to investigate how thiol/disulfide homeostasis changes in patients with pulmonary thromboembolism (PTE).

Material and Methods: In our study, serum thiol/disulphide levels of 45 patients diagnosed with PTE and 50 healthy controls were measured with a new automated spectrometric method and the results were compared statistically.

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: As a result of our study, we saw that the oxidant-antioxidant balance shifted in the oxidative direction. The disulfide / native thiol ratio can be considered as an oxidative stress parameter in PTE. We think that disruption of the thiol-disulfide balance may be important in the pathogenesis of PTE.

Keywords: Pulmonary thromboembolism, thiol–disulphide homeostasis, oxidative stress.

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 of the 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, which contains a sulfhydryl (-SH) group in its structure, is an organic molecule that 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 and methionine, are the primary targets of reactive oxygen species (ROS). ROS tend to 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 the studies performed, they found native thiol and total thiol values to be lower than the control group in cases such as pneumonia and gastric 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 in preventing oxidative tissue damage (7). There are various biochemical markers that show oxidative stress (OS) and inflammation. One of them is the dynamic thiol/disulfide equilibrium. Thiol/disulfide homeostasis (TDH) plays a critical role in many cellular activities, such as antioxidant protection, detoxification, apoptosis, cell growth, enzyme activities and signal transduction (7,14). Thiols, which make up an important part of the total antioxidants in the body, play an important role in the body's defense against reactive oxygen species. Plasma thiols are playing a physiological role as antioxidants and scavenge free radicals through a variety of mechanisms (15). In this study, we tried to determine how the thiol disulfide balance changed in PTE cases and examined its usability as a new biomarker.

MATERIAL AND METHODS

We performed this study regarding 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. A total of 95 people, including 45 patients over the age of 18 who were diagnosed with PTE after applying to the Chest Diseases Polyclinic or Emergency Service of our hospital, and 50 healthy volunteers over the age of 18 with no history of disease and normal physical examination findings 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 collected from patient and control groups were centrifuged at 1500 rpm for 10 minutes to obtain serum. The separated serum was immediately placed in Eppendorf tubes and the samples were stored at -80°C until use.

Measurement of thiol/disulfide parameters was made by the method developed by Erel and Neselioğlu (7). After measuring the native thiol, total thiol and disulfide values, disulfide/native thiol, disulfide/total 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 this parameters. Independent 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. Cross-tabs 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).

Table 1. Evaluation of patients (PTE) and control group in terms of gender

	Patient	Control	P
Female	25 (%55.6)	26 (%52)	0.73
Male	20 (%44.4)	24 (%48)	

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)

Table 2. Evaluation of PTE (Patient) and control group in terms of thiol and disulphide levels.

	Group	N	Average± SD	P
Native thiol (SH) (µmol/L)	Control	50	301.62 ± 58.34	0.000
	Patient	45	228.65 ± 47.96	
Total thiol (TT) (µmol/L)	Control	50	329.76 ± 61.65	0.000
	Patient	45	271.73 ± 48.33	
Disulphide (SS) (µmol/L)	Control	50	14.07 ± 4.04	0.000
	Patient	45	21.54 ± 5.19	
%Disulphide/Native thiol (SS/SH)	Control	50	4.81 ± 1.61	0.000
	Patient	45	9.84 ± 3.13	
%Disulphide/Total thiol (SS/TT)	Control	50	4.35 ± 1.32	0.000
	Patient	45	8.11 ± 2.17	
%Native thiol/Total thiol (SH/TT)	Control	50	91.29 ± 2.64	0.000
	Patient	45	83.77 ± 4.35	

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.

REFERENCES

1. Stein PD, Hull RD, Saltzman HA, Pineo G. Strategy for diagnosis of patients with suspected acute pulmonary embolism. Chest. 1993;103(5):1553-9.
2. Guidelines on diagnosis and management of acute pulmonary embolism. Task Force on Pulmonary Embolism, European Society of Cardiology. Eur Heart J 2000;21:1301-2.
3. Giuntini C, Di Ricco G, Marini C, Melillo E, Palla A. Epidemiology. Chest. 1995;107(1):3-9.
4. Wiener RS, Schwartz LM, Woloshin S. Time trends in pulmonary embolism in the United States: evidence of overdiagnosis. Archives of internal medicine. 2011;171(9):831-7.
5. Pollack CV, Schreiber D, Goldhaber SZ, et al. Clinical characteristics, management, and outcomes of patients diagnosed with acute pulmonary embolism in the emergency department: initial report of EMPEROR (Multicenter Emergency Medicine Pulmonary Embolism in the Real World Registry). Journal of the American College of Cardiology. 2011;57 (6):700-6.
6. Wells PS, Anderson DR, Rodger M, et al. Derivation of a simple clinical model to categorize patients' probability of pulmonary embolism: increasing the model utility with the SimpliRED d-dimer. Thromb Haemost 2000; 83: 418-9.
7. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis, Clin Biochem 2014 Dec;47(18):326-32.
8. Parlak ES, Alisik M, Hezer H, Karalezli A, Hasanoglu HC, Erel O. Evaluation of dynamic thiol/disulphide redox state in community-acquired pneumonia. Saudi Med J. 2018;39(5):495-9.
9. Hizal M, Sendur M, Bilgin B, et al. Evaluation of dynamic serum thiol/disulphide homeostasis in locally advanced and metastatic gastric cancer, Journal of Oncological Sciences, 2018; 4:1-4.
10. Emre S, Demirseren DD, Alisik M, Aktas A, Neselioglu S, Erel O. Dynamic thiol/disulphide homeostasis and effects of smoking on homeostasis parameters in patients with psoriasis, Cutaneous and Ocular Toxicology, 2017, 36:4, 393-6.
11. Kalkan G, Emre S, Alisik M, Aktaş A, Baran P. Dynamic thiol/disulphide homeostasis in patients with lichen planus. J Clin Lab Anal. 2018 ;3:e22642.
12. Fidan F, Alkan BM, Uğurlu FG, et al. Dynamic Thiol/Disulphide Homeostasis in Patients With Fibromyalgia. Arch Rheumatol. 2017;32(2):112-7.

13. Dikensoy O, Celik N, Kul S, Gogebakan B, Bayram H. Ischemia modified albumin in the differential diagnosis of pleural effusions, Respir Med. 2011;105(11):1712-7.
14. Jones DP, Liang Y. Measuring the poise of thiol/disulphide couples in vivo, Free Radical Biology and Medicine 47(10) (2009) 1329-1338.
15. Ozyazici S, Karateke F, Turan U, et al. A novel oxidative stress mediator in acute appendicitis: thiol/disulphide homeostasis, Mediators of inflammation. 2016; 2016 : 6761050.
16. Carson JL, Kelley MA, Duff A, et al. The clinical course of pulmonary embolism. N Engl J Med 1992;326:1240–5.
17. Perrier A. Noninvasive diagnosis of pulmonary embolism. Haematologica 1997;82:328-31.
18. Demir E, Giden R, Sak Zafer H.A. Demir Giden Z. Thiol-disulphide homoeostasis as a novel oxidative stress biomarker in lung tuberculosis patient. Int J Clin Uygulaması . 05 January 2021 ;e13998. https://doi.org/10.1111/ijcp.13998.
19. Parlak ES, Alisik M, Karalezli A, et al.. Are the thiol/disulphide redox status and HDL cholesterol levels associated with pulmonary embolism? Thiol/disulphide redox status in pulmonary embolism. Clin Biochem. 2017 Dec;50(18):1020-4.
20. Topuz M, Kaplan M, Akkus O, et al. The prognostic importance of thiol/disulphide homeostasis in patients with acute pulmonary thromboembolism. Am J Emerg Med. 2016 Dec;34(12):2315-9.