Research Article

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Evaluation of thiol disulphide levels in patients with pulmonary embolism

Pulmoner Emboli Hastalarında Tiyol Disülfid Düzeylerinin Değerlendirilmesi

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Abstract

Objectives: Maintaining the thiol-disulphide balance is essential for antioxidant defense and apoptosis. The study aimed to evaluate of thiol-disulphide levels in patients with pulmonary embolism (PE).

Methods: The study included a total of 97 participants: 48 patients and 49 healthy individuals. Serum native thiol (NT), total thiol (TT) and disulphide (DS) levels (μmol/L) were measured using the novel spectrophotometric method.

Results: We found significantly lower levels of NT (195.44 ± 83.3 μmol/L), DS (20.42 ± 9.20 μmol/L) and TT (236.26 ± 90.66 μmol/L) in patients with PE compared with the healthy controls (304.42 ± 62.13, 24.33 ± 8.09 and 353.07 ± 63.58 μmol/L respectively). Patients with PE had lower serum albumin levels (3.11 ± 1.03 g/dL) and higher ischaemia modified albumin (IMA) levels (0.82 ± 0.16 g/dL) compared with the controls (3.89 ± 0.52 and 0.67 ± 0.15 g/dL respectively). Serum albumin levels in patients were strongly correlated with plasma IMA (r=−0.737; p<0.001), NT (r=0.786; p<0.001) and TT levels (r=0.841; p<0.001). Serum NT levels were strongly correlated with serum TT levels (r=0.981; p<0.001) in patients.

Conclusions: This study found lower TT, NT and DS levels in patients with PE than in the control group. Our study revealed that thiol-disulphide homeostasis could be altered during PE and further studies are needed to be used as prognostic markers for hospital mortality.

Keywords: antioxidant effect; homeostasis; oxidative stress; pulmonary embolism; ROC analysis; thiols.

ÖZ


Yöntem: Çalışmaya toplam 97 katılmıcı dahil edildi: 48 hasta ve 49 sağlıklı birey. Serum native tiyol (NT), total tiyol (TT) ve disülfid (DS) seviyeleri (μmol/L) yeni spektrofotometrik yöntem kullanılarak ölçüldü.

Bulgular: PE’li hastalarda sağlıklı kontrollere göre (sırasıyla, 304.42±62.13, 24.33±8.09 ve 353.07±63.58 μmol/L) anlamlı derecede daha düşük NT (195.44±83.3 μmol/L), DS (20.42±9.20 μmol/L) ve TT (236.26±90.66 μmol/L) seviyeleri bulundu. PE’li hastalarda kontrollere kıyasla (sırasıyla 3.89±0.52 ve 0.67±0.15 g/dL) serum albümin seviyeleri (3.11±1.03 g/dL) daha düşük ve iskemi modifiye albümin (IMA) seviyeleri (0.82±0.16 g/dL) daha yüksekti. Hastalardaki serum albümin seviyeleri, plazma IMA seviyeleri (r=−0.737; p<0.001), NT (r=0.786; p<0.001) ve TT seviyeleri (r=0.841; p<0.001) ile güçlü korelasyon gösterdi. Hastalarda serum NT seviyeleri,
Introduction

PE is a sudden occlusion of a pulmonary artery. This blockage is usually caused by a blood clot coming from a vein in the leg to the lung. PE is a widespread disease with non-specific symptoms that has a potentially high risk of mortality and morbidity. In the United States, 500,000 to 600,000 individuals are diagnosed with PE per year. Many fatal pulmonary embolisms remain undiagnosed because of the lack of routine post-mortem examination, resulting in an underestimation of PE incidence [1–4].

Two of the most commonly used scoring systems to define PE are the Wells score and the Geneva clinical probabilities. The current standard radiological examination is computed tomography pulmonary angiography (CTPA). Physicians often face diagnostic challenges and CTPA needs to be utilised, even low probability cases [1, 2, 5]. PE risk factors include obesity, immobilisation, cigarette use, cancer, surgery, pregnancy, oral contraceptives or hormone replacement therapies [1, 6]. PE accompanied by shock and heart failure further increases the risk of mortality [7].

Reactive oxygen species (ROS) are unstable species capable of initiating oxidation owing to the existence of unpaired valency shell electrons [1, 8, 9]. ROS is a native by-product of normal oxygen metabolism and plays a significant physiological role in signal transduction. However, they can contribute to the disease mechanisms through the dysregulation of oxidative damage to cellular macromolecules [10, 11]. Smoking, asthma, chronic inflammation are associated with an oxidant-antioxidant imbalance that can be identified in blood [11, 12]. A balance between oxidation and antioxidation is critical in respiratory tissues in the physiopathology of COPD and PE [11, 13]. Experimental evidence [14] indicates the oxidative stress of lung tissue in laboratory animals with PE [15].

As a result of pulmonary circulatory failure due to PE, circulatory instability, hypoperfusion, hypoxia and ischemia occur. All these conditions cause oxidative stress in the organism. Oxidative stress occurs when there is an instability between ROS production and the body’s ability to readily detoxify the reactive mediators or easily repair the resultant damage [14–16]. Oxidative stress and associated ROS can damage DNA, lipids, and proteins. In events that may cause an acute ischemic condition such as acidosis and hypoxia, the N-terminal end of the albumin is modified and its capacity to bind transition metals such as cobalt and copper is reduced. This modified form of albumin is called Ischemia Modified Albumin (IMA) and accepted as a biochemical test of oxidative stress. When cells of a healthy body are exposed to ROS, antioxidant mechanisms are activated. Therefore, an oxidant-antioxidant balance shifts favouring the oxidant. Thiols contribute to an increase in the amount of antioxidants and their contribution to defense against ROS is great [11, 16, 17].

In response to the damages of reactive oxygen radicals, many different mechanisms in the body control them. These systems complement each other as they play a role in different cells and different free radicals. In biological systems, thiols are bound to albumin and amino acids and contain sulphur [18, 19]. Thiols become oxidised reacting with reactive oxygen radicals thus removing them to prevent tissue damage and the resultant disulphide bonds can be diminished back to thiol groups. So, the thiol-disulphide balance is continued [20]. Oxidative stress can be assessed indirectly by measuring thiol-disulphide levels [13]. The topic of thiol-disulphide balance has garnered interest lately.

Dynamic thiol-disulphide balance can represent the oxidative status of the organism. Studies are showing that thiol disulphide balance is impaired under conditions of increased oxidative stress (pulmonary diseases and pulmonary embolism). Because pulmonary embolism is a disease with high mortality, early diagnosis and treatment are very important in terms of prognosis. Thiol-disulphide balance is a current marker of interest in determining changes in oxidative stress. Compared with conventional oxidative stress markers, the new assay improved by Dr. Erel and Dr. Neselioglu provides a cost-effective, practical and fully automated spectrophotometric analysis that can detect plasma thiol-disulphide homeostasis. Therefore, we purposed to assess the impact of thiol-disulphide levels in the etiopathogenesis of PE, considering that oxidative stress may affect prognosis in PE.
Material and methods

Study population

The study group included 48 patients diagnosed with PE admitted to the Department of Pulmonary Medicine, and 49 healthy volunteers without any known illness. It was planned to study the samples taken during the routine treatment procedures of the individuals and not to perform any further invasive procedure barring routine applications. In our study, the lack of patient consents who had heart valve disease, chronic renal failure, malignancy, diabetes, and who received vitamin and antioxidant supplements were excluded from the study.

Biochemical analyses

Blood samples were collected within 24 h of the first hospitalization of patients diagnosed with PE and after at least 8 h of fasting. Samples were centrifuged at 3000 rpm for 15 min (following coagulation) and their serums were separated. The serums were then stored at −80 °C until required for analysis. IMA and thiol measurements were performed simultaneously with the same samples. In the IMA measurements, we used the measurement rapid colorimetric method of Bar-Or et al. [21]. Dynamic disulphide bonds are reduced to free functional thiol groups by sodium borohydride and methanol solution. The unused sodium borohydride was consumed and removed with formaldehyde to prevent the decline of DTNB (5,5’-dithiobis(2-nitrobenzoic) acid), and all of the thiol groups including reduced and native thiol groups were determined after the reaction with DTNB spectrophotometrically at 412 nm. The total thiol amount of samples was measured using the modified Ellman reagent. The half value of the difference between total thiol and native thiol amounts gave the disulphide bond amount. After detection of the native thiol and disulphide amount, the ratio of disulphide/total thiol (DTT), native thiol/total thiol (NTT) and disulphide/native thiol (DTN) was calculated (%). Thiol-disulphide homeostasis parameters were measured using a novel spectrophotometric method [22]. Recovery in thiol disulphide measurement was 98–101%. The upper limit of the linearity for the native thiol measurement was 4,000 µM and for the disulphide measurement was 2000 µM. The detection limit was 2.8 µM.

Statistical analyses

SPSS 18.0 package program was used to analyze the data in this study. Statistical significance was considered with a probability of p<0.05. The results as mean and standard deviation are given in the table. Kolmogorov–Smirnov and Shapiro–Wilk test analysis of non-normal distributed variables was performed using non-parametric methods. Mann–Whitney U test was applied for variables with a non-normal distribution. The presence of a correlation between the groups was analyzed with the Spearman correlation test. Receiver Operating Characteristics (ROC) curve was used for the calculation of the sensitivity and specificity of total thiol, native thiol and IMA.

Results

We found significantly lower levels of albumin (3.11±1.03 g/dL), native thiol (195.44±83.3 µmol/L), disulphide (20.42±9.20 µmol/L) and total thiol (236.26±90.66 µmol/L) in patients with PE compared with healthy controls (3.89±0.52, 304.42±62.13, 24.33±8.09, and 353.07±63.58 µmol/L, respectively). Serum IMA levels (0.82±0.16 g/dL) were higher compared with controls (0.67±0.15 g/dL) (Table 1).

Serum albumin levels in patients with PE were strongly correlated with plasma IMA (r=−0.737; p<0.001), native thiol (r=0.762; p<0.001) and total thiol (r=0.822; p<0.001).

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Some important correlation graphs are given in Figures 1 and 2. Diagnostic performance using ROC plots curve indicated that total thiol cut-off values of 296.5 or below could predict PE with 71.4% sensitivity and 70% specificity for the area under curve (AUC=0.874; 95% confidence interval 0.772–0.923) and native thiol cut-off values of 256.65 or below could predict PE with 75.5% sensitivity and 82% specificity (AUC=0.838; 95% confidence interval 0.760–0.917) (Figure 3).

Table 1: Plasma thiol-disulphide levels of PE and control group.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PE (n=48) (mean ± SD) (minimum–maximum)</th>
<th>Controls (n=49) (mean ± SD) (minimum–maximum)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dL)</td>
<td>3.11 ± 1.03 (0.35–4.54)</td>
<td>3.89 ± 0.52 (2.13–4.59)</td>
<td>0.000*</td>
</tr>
<tr>
<td>IMA (g/dL)</td>
<td>0.82 ± 0.16 (0.46–1.3)</td>
<td>0.67 ± 0.15 (0.26–1.20)</td>
<td>0.000*</td>
</tr>
<tr>
<td>Native thiol (µmol/L)</td>
<td>195.44 ± 83.3 (40.2–371.4)</td>
<td>304.42 ± 62.13 (201.5–461.6)</td>
<td>0.033*</td>
</tr>
<tr>
<td>Disulphide (µmol/L)</td>
<td>20.42 ± 9.20 (0.8–44)</td>
<td>24.33 ± 8.09 (7.1–48.8)</td>
<td>0.009*</td>
</tr>
<tr>
<td>Total thiol (µmol/L)</td>
<td>236.26 ± 90.66 (65.8–413.7)</td>
<td>353.07 ± 63.58 (238.6–514.7)</td>
<td>0.004*</td>
</tr>
<tr>
<td>Disulphide/native thiol (%)</td>
<td>12.28 ± 7.63 (0.62–37.76)</td>
<td>8.35 ± 3.55 (2.91–20.31)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Disulphide/total thiol (%)</td>
<td>9.35 ± 4.32 (0.61–21.51)</td>
<td>7.01 ± 2.44 (2.75–14.44)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Native thiol/total thiol (%)</td>
<td>81.30 ± 8.64 (56.98–98.78)</td>
<td>85.98 ± 4.87 (71.11–94.50)</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

*Significant in p<0.05 level.
Diagnostic performance using ROC plots curve indicated that IMA cut-off values of 0.932 or below could predict PE with 95.9% sensitivity and 26.5% specificity (AUC=0.794; 95% confidence interval 0.703–0.885) (Figure 4).

Discussion

Protective mechanism against oxidative cell damage and oxidative stress is the thiol group of the cellular protein [15, 23]. Oxidative stress has been a topic of interest for clinicians for a long time. Notably, it has a critical role in the development of various diseases, like type 1 DM, cancer, atherosclerosis, and HT. Studies have shown that the etiopathogenesis of the disease changes with increased oxidative stress [12, 20]. Accordingly, clinical conditions that influence oxidant-antioxidant balance were excepted from our study. The measurement of thiol-disulphide homeostasis is crucial in explaining the impacts of oxidative stress and for evaluating disease.

Our study found that albumin, native thiol, and total thiol levels were remarkably reduced in patients with PE which was remarkably related to PE patients. When ROC analysis is analyzed, we can say that total thiol and native thiol have higher sensitivity-specificity according to IMA. The sensitivity and specificity of native thiol for this prediction quite high. Another study revealed that thiol-disulphide homeostasis can be changed during PE and associated with worse haemodynamic parameters and can serve as a prognostic marker for hospital mortality [25].
In our study, the DNT and DTT ratio showing oxidation was significantly higher in PE. There was a negative correlation with DNT and DTT ratio in patients with PE. The increase in DNT and DTT ratios and the decrease in the NTT ratio indicate that the thiol-disulphide balance shifts to the disulphide side, thereby indicating that increased oxidative stress result in PE. In cases where oxidative stress increases, the amount of thiol decreases. Oxidative stress was high, whereas antioxidative parameters were reduced in patients with PE. Strong correlations between the activity of the PE and thiol-disulphide homeostasis indicate that homeostasis may affect the progress of the disease. Parlak et al. reported that the DNT rate was significantly higher in patients with PE [24]. In our study, the rates of DNT and DTT were found to be high in the patient group and our study results are consistent with the literature.

Albumin is one of the most important and most effective antioxidants in plasma. Plasma proteins (especially serum albumin) are very sensitive to oxidation due to free –SH groups. Albumin has an essential role in total antioxidant capacity and its levels reduced because of fast impairment during inflammation and oxidative stress [13, 26]. Decreased albumin levels and increased IMA levels indicate that antioxidant capacity shifts towards the oxidant side. We did not find any other studies evaluating IMA levels and thiol-disulphide levels in patients with PE. In this study showed that albumin levels reduced and IMA levels increased in PE. These findings provide a context for understanding the role of the dynamic thiol-disulphide homeostasis and its relation with IMA, an indicator of oxidative stress, in PE patients.

Recently, studies on thiol-disulphide homeostasis have been popular and have gained importance. Kundi et al. [27] expressed that the thiol-disulphide rate riced in acute myocardial infarction, and this rate may enable detection of acute myocardial damage. Koprivica et al. [28] demonstrated the role of oxidative stress in the improvement of endothelial dysfunction in different types of the acute coronary syndrome (ACS). Ates et al. [29] indicated that thiol oxidation increased in type 1 diabetics and this increase was proportional to the severity of the disease. Furthermore, there are some studies evaluating thiol-disulphide homeostasis in patients with preeclampsia and HT [14, 29, 30]. Our results showed a raised thiol-disulphide ratio in patients with PE, which was statistically important, indicating that the decrease in thiol may be crucial factor in the etiology of PE. These results indicate that we can benefit from thiol-disulphide balance in the future stages in estimating the course of the disease by making larger studies.

**Limitations of the study**

This study had several restrictions. First, there were few patients. Second, despite excluding participants taking supplemental vitamins, no standardisation could be made regarding the antioxidant content of the daily diet of the participants. Third, different antioxidant factors such as lipid hydroperoxide were not measured. In addition, although we exclude diseases where oxidant-antioxidant balance shifts to antioxidant side such as diabetes, malignancy, hypertension, kidney diseases etc., there is a possibility that people who have not been diagnosed in terms of these diseases are in our control group. For future studies, it would be more valuable to carry out a study with a group of patients who would have a clinical symptom similar to the disease, except for the control group.

**Conclusion**

The measurement of thiol-disulphide homeostasis is crucial for elucidating the effects of oxidative stress and evaluating disease. This study indicated that the oxidative/anti-oxidative balance shifted to the oxidative side in patients with PE. Our study revealed that thiol-disulphide homeostasis can be altered during PE. It is easily accessible, simple calculated and relatively inexpensive. The results can be supported by studies involving more patients with PE and larger studies can be performed to use thiol disulphide as a prognostic marker.
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**Competing interest:** There is no conflict of interest in this study.

**Informed consent:** Informed consent was obtained from all individuals included in this study.

**Ethics approval:** Ethics approval was obtained from Selçuk University, Health Sciences Institute, Konya, Turkey (Ethics Committee No: 03/01/2018).

**References**
