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Importance of Oxidative Stress in Pathogenesis and its Value in Diagnosis of Pulmonary Embolism Patients

Oksidatif Stresin Patogenezdeki Önemi ve Pulmoner Embolili Hastaların Tanısındaki Değeri

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Abstract

Objective: Symptoms related to pulmonary embolism (PE) can be nonspecific and difficult to discriminate from many other clinical situations. The purpose of this study was to evaluate the changes in oxidative stress in patients with acute PE to find t the pathophysiology of the mechanism and to assess the value of oxidative stress in the diagnosis of pulmonary embolism.

Material and Methods: A total of 27 consecutive patients confirmed as PE by computerised tomography (CT) results were retrospectively included in the study. Thirty healthy volunteers were included as the control group. The serum antioxidative status was evaluated by measuring total antioxidant status (TAS) levels in patients with PE and in healthy individuals. The serum oxidative status was evaluated by measuring total oxidant status (TOS). Then the oxidative stress index (OSI) was also calculated.

Results: The male/female ratio of patients was 15/12. Mean age was 59.00±21.01 years. TOS and OSI levels increased in the patient group compared to the control group (respectively, 14.12±9.82 µmol H_2O_2 equivalent/L vs. 7.83±2.21 µmol H_2O_2 equivalent/L, p=0.001; 0.66±0.49 arbitrary unit vs. 0.37±0.09 arbitrary unit, p=0.002). But no difference was found in TAS levels of the two groups (p>0.05).

Conclusion: Oxidative stress parameters play an important role in the pathophysiology of PE and it should be considered as a diagnostic marker together with other diagnostic tools. *(JAEM 2012; 11: 19-22)*

Key words: Pulmonary embolism, diagnosis, oxidative stress, total oxidant status, total antioxidant status

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Özet

Amaç: Pulmoner emboli ile ilişkili semptomlar nonspesifik ve diğer birçok klinik durumlardan ayırtedilmesi zor olabilir. Bu çalışmanın amacı, mekanizmanın patofizyolojisini bulmak için pulmoner emboli tanısında oksidatif stres değerini değerlendirmek ve akut PE tanısı alan hastalarda oksidatif stres değişikliklerini saptamaktı.

Gereç ve Yöntemler: Bilgisayarlı tomografi ile PE tanısı doğrulanan 27 hasta retrospektif olarak çalışmaya dahil edilmiştir. Otuz sağlıklı gönüllü kontrol grubu olarak alınmıştır. Serum antioksidan durumu PE hastaları ve sağlıklı gönüllülerde total antioksidan durum (TAS) düzeyleri ölçülerek değerlendirildi. Serum oksidatif durumu total oksidatif durum (TOS) ölçülerek değerlendirildi. Sonra da oksidatif stres indeksi (OSI) hesaplandı.

Bulgular: Hastaların erkek/kadın oranı 15/12 idi. Yaş ortalaması 59.00±21.01 yıldı. Kontrol grubu ile karşılaştırıldığında TOS ve OSI düzeyleri hasta grubunda arttı (sırasıyla 14.12±9.82 µmol H_2O_2 equivalent/L vs. 7.83±2.21 µmol H_2O_2 equivalent/L, p=0.001; 0.66±0.49 arbitrary unit vs. 0.37±0.09 arbitrary unit, p=0.002). Ama her iki grupta TAS düzeylerinde fark yoktu (p>0.05).

Sonuç: Oksidatif stres parametreleri PE nin patofizyolijisinde önemli rol oynar ve diğer tanı araçlarının yanında tanısal bir belirteç olarak düşünülebilir. *(JAEM 2012; 11: 19-22)*

Anahtar kelimeler: Pulmoner emboli, tanı, oksidatif stres, total oksidan durumu, total antioksidan durumu

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Introduction

Pulmonary embolism (PE) is the third most important cause of mortality from cardiovascular disease, after myocardial infarction and cerebrovascular stroke (1).

PE is a dramatic and life-threatening complication of deep venous thrombosis (DVT). The primary mechanism in the pathophysiology of DVT consists of venous stasis, vascular injury, and hypercoagulability, which are known as Virchow's triad. DVT patients may also have individual risk factors such as a history of thromboembolism, cancer, advanced age, or genetic properties linked to hypercoagulation (2).

Patients with PE present with nonspecific symptoms including chest pain, palpitations, breathing difficulties and haemoptysis. Timely diagnosis and treatment as well as an adequate prophylaxis can significantly reduce morbidity and mortality rates (3, 4).

The research of risk factors, development of clinical probability algorithms, diagnostic evaluation, characterization of new therapeu-

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tic choices and thromboprophylaxis recommendations requires particular attention in PE patients (4).

Oxidative stress arises from an imbalance between the production of ROS and the biological system's ability to readily detoxify the reactive intermediates (5). The presence of oxidative stress in patients with PE was shown in previous studies (6).

The aim of this study is to evaluate the alterations in oxidative stress in patients with acute PE, to determine the pathophysiology of the mechanism and to investigate the value of oxidative stress in the diagnosis of PE.

Meterial and Methods

Study population and protocol

The study was performed in 2010. It was approved by the institution's ethics committee. All patients with suspected PE admitted to the emergency department of our hospital were evaluated. Patients were included in the study if the diagnosis of PE was confirmed by CT results.

The diagnosis of acute PE was confirmed in 27 patients and the remaining unconfirmed ones were excluded.

Routine diagnostic tools for PE included D-dimer, arterial blood gases, electrocardiography and CT evaluation. Data were collected retrospectively for confirmed cases of PE. The control group included 30 healthy individuals who volunteered to participate in this study. They had no condition that would affect the study parameters. On admission to the emergency department, venous blood was drawn into blood tubes from PE patients, and then confirmed by CT, and serum was separated from the cells by centrifugation at 1500 g for 10 min, the serum samples were stored at -80°C until analysis. The serum oxidative status was evaluated by measuring TOS and serum antioxidative status was evaluated by measuring TAS levels in patients with PE. The percent ratio of TOS to TAS level was accepted as OSI.

Similar studies were also performed in the control group. All these data were compared between PE patients and healthy control individuals.

Determination of Serum Total Oxidant Status (TOS) Levels

TOS levels were measured using commercially available kits (Rel assay, Turkey). In the new method, oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromoles hydrogen peroxide equivalent per litre (μmol H_2O_2 equivalent/L) (7).

Determination of Serum Total Antioxidant Status (TAS) Levels

TAS levels were measured using commercially available kits (Rel assay, Turkey). The novel automated method was based on the bleaching of the characteristic color of a more stable ABTS (2.2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants. The assay had excellent precision values, which were lower than 3%. The results were expressed as mmol Trolox equivalent/L (8).

Calculation of oxidative stress index (OSI)

The ratio of TOS to TAS was accepted as the OSI. For calculation, the resulting unit of TAS was converted to μmol/L, and the OSI value was calculated according to the following formula: OSI (arbitrary unit)=TOS (μmol H2O2 equivalent/L)/TAS (μmol Trolox equivalent/L) (9-11).

Statistics

For statistical evaluation, we used the software package SPSS 15.0 and a probability value of less than 0.05 was accepted as statistically significant. As the data were normally distributed and independent, statistical analysis was performed using Student's t-test when comparing groups. The results are given as the mean±standard deviation (SD).

Results

A total of twenty seven consecutive patients confirmed as PE by computerised tomography (CT) results were retrospectively included in the study. Thirty healthy volunteers were included as the control group. The male to female ratio of groups 1 and 2 were 15/12 (55.5% male, 44.5 female) and 19/11 respectively. Mean age was 59.00±21.01 years.

Risk factors, symptoms, clinical signs, and common laboratory findings in PE on admission in the emergency department were shown in (Table 1).

TOS and OSI levels increased in the patient group when compared to the control group (respectively, 14.12±9.82 μmol H_2O_2 equivalent/L vs. 7.83±2.21 μmol H_2O_2 equivalent/L, p=0.001; 0.66±0.49 arbitrary

Table 1. Symptoms, clinical signs, and common laboratory findings in PE

		frequency
Risk factors	History of DVT	20%
	Recent surgery or immobilization	22.2%
Symptoms	Dyspnea	94%
	Pleuritic pain	36.8%
	Haemoptysis	5.3%
	Syncopal episode	10.5%
Clinical signs	Tachypnea (>20/min)	10.5%
	Tachycardia (>100/min)	10.5%
	Signs of deep venous thrombosis	36.8%
Radiological findings	Atelectasis	47.4%
	Pleuritic effusion	44.4%
	Peripheral opacification (infarct)	22.2%
	Raised hemidiaphragm	36.8%
	Reduced pulmonary vascularisation	33.3%
Blood gases	Hypoxemia	73.7%
Electrocardiogram	Right ventricular hypertrophy	47.4%

unit vs. 0.37±0.09 arbitrary unit, p=0.002) (Figure 1, 2). However, TAS levels in both groups showed no difference, (p>0.05) (Figure 3). These values for all parameters are presented as mean±SD in (Table 2).

Based on CT results, 21.1% of patients were massive PE, the remaining ones were submassive PE. TOS and OSI levels in massive PE patients were higher than in submassive PE patients, but statistically there were no significant differences between the two groups, 18.05±14.85 μmol H_2O_2 equivalent/L vs. 13.32±8.70 μmol H_2O_2 equivalent/L; 0.87±0.86 arbitrary unit vs. 0.62±0.38 arbitrary unit respectively).

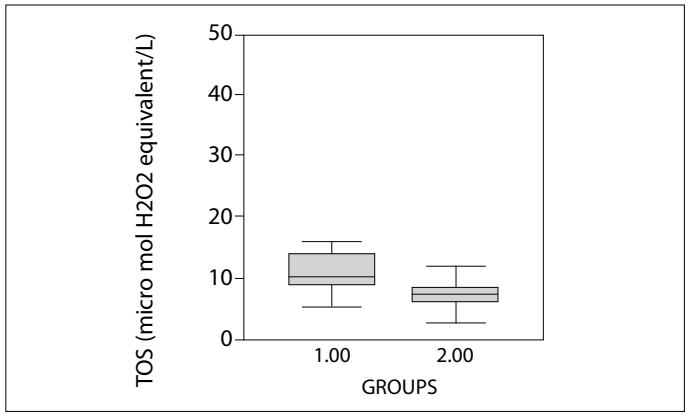

Figure 1. Differences in TOS levels between pulmonary embolism patients (Group 1) and control group (Group 2)

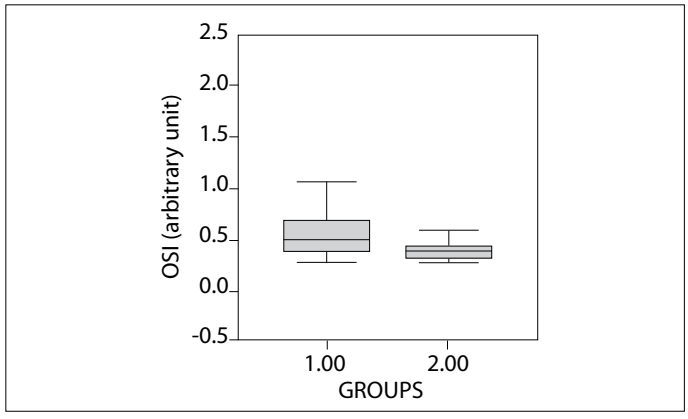

Figure 2. Differences in OSI levels between pulmonary embolism patients (Group 1) and control group (Group 2)

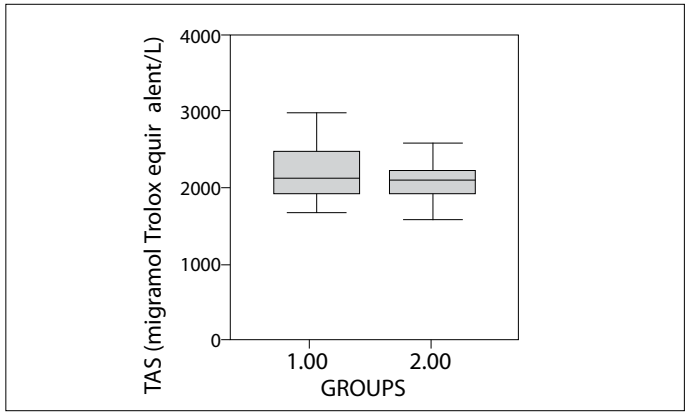

Figure 3. TAS levels in pulmonary embolism patients (Group 1) and control group (Group 2)

Discussion

In this study, levels of TOS and OSI as predictors of oxidative status in PE patients were significantly higher than those of healthy individuals., but there were no statistical differences in TAS levels for the two groups. These results show that the oxidative/antioxidative balance shifted towards the oxidative status, namely increased oxidative stress was present in patients with PE compared to healthy control individuals.

PE is a frequently occurring, acute, and potentially fatal condition. Numerous risk factors for PE, both inherited and acquired, have been identified (12). Early diagnosis and adequate treatment of PE is very important, because the mortality rate of untreated PE is 30% compared to 8% when treated. The signs and symptoms of PE are nonspecific and can not be distinguished from many other diseases (13-15). Tachycardia, chest pain, cough, unexplained loss of consciousness, and/or haemoptysis raise the suspicion of PE, while hypoxemia, haemodynamic instability, syncopal episode and/or cyanosis are characteristic of massive PE (15).

Regarding previous researches, pulmonary macro- or microobstruction, depending on embolus size, together with reactive arterial vasoconstriction is the underlying pathophysiology of PE. Reactive arterial vasoconstriction leads to pulmonary hypertension. Acute pulmonary hypertension may cause right ventricular failure (acute cor pulmonale) and finally cardiogenic shock (14, 16). In addition to all these events, the existence of oxidative stress in the pathophysiology of PE was shown by previous studies (6, 17-19).

Only about 20% of individuals with suspected PE will have the diagnosis confirmed, therefore, the diagnostic modality for PE should comprise safe, efficient, and noninvasive methods. The first step in the approach to diagnosis of patients with suspected PE is to determine the clinical probability and to perform a D-dimer test. PE can be excluded in patients with a low, intermediate or unlikely clinical probability and a normal D-dimer test. Additional imaging is required for those with a high clinical probability or a positive D-dimer test. CT pulmonary angiography or ventilation-perfusion scintigraphy, followed by additional testing is the next step when test results are nondiagnostic (12, 20).

A combination of multi-slice CT and negative D-dimers essentially rules out PE (15, 21).

Transthoracic echocardiography is considered useful in the diagnosis of pressure overload in the right chambers. Thus, it is of particular use in identifying patients with a large PE, in whom the pressure of the pulmonary circulation is elevated (90% sensitivity) (15).

CT pulmonary angiography has become a first-line imaging test for evaluation of PE because of its high accuracy, ease of use, and

Table 2. TOS, TAS and OSI levels between patient and control groups (mean± SD)

Parameters	Group	mean± SD	p
TOS	patient	14.12±9.82	0.001
(μmol H_2O_2 equivalent/L)	control	7.83±2.21	
TAS	patient	2194.21±497.31	>0.05
(μmolTrolox equivalent/L)	control	2082.28±283.31	
OSI	patient	0.66±0.49	0.002
(arbitrary unit)	control	0.37±0.09	

ready availability. However, there are some restrictions in the application of this test due to exposure of ionizing radiation and usage of contrast material (22, 23). To solve these limitations, SPECT V/Q and MR are recommended (24). However, we think that to obtain the mentioned diagnostic tools is not easy in most emergency cases. In our study, in patients presenting with clinically suspected PE, oxidative stress was associated with confirmed PE. These findings suggest that oxidative stress is involved in the pathogenesis of the PE. Therefore if PE is suspected in the ED, determining of oxidative stress parameters may be an useful adjunct to laboratory and clinical findings in the diagnosis of PE.

ROS are produced regularly in cells with normal metabolism. On the other hand, cells are able to cope with protecting themselves from the destructive potential of oxygen radicals in normal physiologic conditions through their own antioxidant mechanisms such as enzyme systems, vitamins, elements, and some antioxidant molecules. There is a critical balance between production and destruction of ROS. When this equilibrium is destroyed, ROS are produced excessively and all tissues are exposed to oxidative injury (25). In previous studies related to PE, the changes of oxidative stress were examined by individual measurements such as SOD and MDA. It was shown that oxidative stress is notably important in PE (17, 26). Our study is the first which investigates TOS, TAS and OSI levels in PE since oxidants and antioxidants have additive effects. Although the concentration of oxidant and antioxidant components can be measured individually, these measurements are time consuming and very expensive and also require sophisticated systems. In addition, it may not accurately reflect the TAS and TOS (27). For this reason, we measured the oxidant status and antioxidant status totally and demonstrated that there is an immediate increase in oxidant status and OSI at the initiation of PE. Oxidative status was evaluated by measuring the TOS and OSI levels. Antioxidative status was evaluated by measuring the TAS in the serum (28).

Based on these results, we suggest that oxidative stress may play an important role in the pathogenesis of PE. Oxidative stress parameters can be defined as a new marker for early identification of PE among patients with suspected PE, submitting to emergency department.

Conflict of Interest

No conflict of interest was declared by the authors.

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