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The role of malondialdehyde in the evaluation of the treatment response in acute pulmonary thromboembolism

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ABSTRACT

Aims: To facilitate the early diagnosis of the related patient group by investigating the level of Malondialdehyde (MDA) in high-risk pulmonary thrombo embolism (PTE). In addition, it was aimed to see the effect of treatment after acute PTE on oxidative stress and to evaluate whether it is associated with the development of chronic thrombus.

Methods: This study was conducted prospectively in 44 patients diagnosed with PTE in single-center. At the time of diagnosis, after 6 months of follow-up and treatment, the MDA levels of the patients were evaluated together with the controlled tests.

Results: MDA level was found to be a determinant in the estimation of high-risk PTE. The average MDA levels of the patients included in the study were found to be 9.64±3.76 before PTE treatment and 4.74±2.48 after treatment. The average MDA level after treatment was found to be 5.15±2.90 in patients whose thrombus persisted, and 3.77±1.99 in patients with resolution. There were significant differences between groups. In addition, It was observed that MDA levels measured at the time of diagnosis and after treatment were higher in patients in whom thrombus resolution could not be achieved after treatment.

Conclusion: MDA level is a guiding parameter in high-risk PTE. In addition, the continuation of oxidative stress in the case of chronic thrombus may be important in the remodeling of the precapillary pulmonary artery.

Keywords: Acute pulmonary thrombo embolism, chronic thrombo embolism, malondialdehyde, oxidative stress

INTRODUCTION

Pulmonary thromboembolism is a disease with high mortality that develops as a result of obstruction of the pulmonary artery (PA) and/or its branches by materials originating from another part of the body (thrombus, air, fat, tumor cells, amniotic fluid, septic materials, etc.).[1] The mean annual incidence of venous thromboembolism (VTE), including deep vein thrombosis (DVT) and PTE, ranges from 23-269/100,000.[2]

In acute PTE cases, "risk classification", which evaluates the risk of 30-day mortality, should be done in order to make the treatment approach correctly. High-risk PTE accounts for less than 5% of all patients with an early hospital mortality of at least 15%.[3]

Thrombus resolution may take up to two months after acute PTE.[4] In a study, patients with PTE were re-evaluated 6 months later and chronic thrombus was reported in approximately 50% of them.[4] While PTE in which large-diameter vessels with no thrombus resolution are involved results in chronic period vascular remodeling, PTE in which small-diameter vessels are involved results in chronic period proliferation and abnormal endothelial function.[5] In addition, the thrombus existing in the vascular bed is organized and plays a key role in the remodeling of the precapillary pulmonary artery by causing changes in the medial and intimal layers of the pulmonary arteries. These changes in the pulmonary artery can result in pulmonary hypertension.

Free radicals are molecules that have one or more unpaired electrons in their final orbital and are therefore highly unstable.[6] In a healthy microorganism, there is a certain balance between the formation and elimination of free radicals. This situation is called oxidative balance. Reactive oxygen products, which are formed as a result of the deterioration of this balance in favor of oxidants, react with the unsaturated bond structures of cholesterol and fatty acids in the cell membrane, forming peroxidation products and causing changes in the chemical and physical structure of the cell membrane.

The most toxic one of the compounds formed as a result of lipid peroxidation are aldehydes. As a result of this reaction, cytotoxic and mutagenic MDA and 4-hydroxynonenal are

formed. MDA has a toxic effect by binding to amino groups of proteins, phospholipids and nucleic acids. It can easily pass through the cell membrane and react with the structural components of DNA. This causes mutagenic, carcinogenic and genotoxic effects.[7] This high reactivity and longevity of MDA have made it one of the most frequently used parameters to measure the peroxidation level.

Oxidative stress develops in the organism as a result of instability, hypoperfusion, hypoxia and ischemia caused by pulmonary thromboembolism.[8] Studies have shown that oxidative stress plays a role in the remodeling of the precapillary pulmonary arteries. Determination of the diagnostic value of oxidative stress in chronic thromboembolic disease (CTED) and chronic thromboembolic pulmonary hypertension (CTEPH) is quite remarkable in the development of new treatment protocols.

The average MDA level after treatment was found to be 5 in patients whose thrombus persisted, and 3.77 in patients with resolution. There were significant differences between groups.

METHODS

This study was designed as a single-center prospective study in a cohort of patients who applied to the Ministry of Health, Health Sciences University, Dışkapı Yıldırım Beyazıt Health Application and Research Center (SUAM) and were diagnosed with PTE after the evaluation of the Chest Diseases Clinic.

This study was approved by the ethics committee with the decision of the University of Health Sciences, Dışkapı Yıldırım Beyazıt SUAM Clinical Researches Ethics Committee dated 16.03.2020 and numbered 84/16 and was carried out following the ethical principles determined by the Declaration of Helsinki.

Patients who refused to participate in the study, had chronic thrombus at the time of diagnosis, had previously been diagnosed with pulmonary hypertension, had uncontrolled metabolic disease, were diagnosed with acute coronary syndrome or acute cerebrovascular disease, had uncured malignancy, or newly diagnosed cancer, had rheumatological disease at the time of acute attack, had interstitial lung disease or chronic obstructive pulmonary disease, patients under 18 years of age and pregnant women were not included in the study. When all these requirements were met, patients who agreed to participate in the study were included in the study.

The study was carried out between 18.03.2020 and 30.06.2021 and a total of 78 patients were included in the study. 10 patients who died, 4 patients who were diagnosed with cancer after the examinations, and 11 patients who did not come to their follow-ups were excluded from the study. 9 patients were not included in the study because under pandemic conditions they wanted to be followed up in another center close to their residence. According to the G-Power analysis, we determined the minimum sample size as 44 patients, and the study was terminated after reaching a sufficient number of patients.

Study Method

Demographic characteristics such as age and gender, smoking histories, comorbidities and symptoms at admission, vital values, imaging tests (chest radiography, CTPA and/or ventilation-perfusion scintigraphy), transthoracic echocardiography (TTE) and compression Doppler ultrasonography (CDU) results, laboratory values (complete blood count, biochemical tests, coagulation tests, d-dimer, pro-BNP, troponin, blood gas values) and medical treatments of patients diagnosed with PTE between the specified dates and meeting the inclusion criteria were analyzed through the in-hospital information management system (HIMS) and the Ministry of Health E-Nabız System. In order to determine the MDA level at the time of diagnosis, 2 tubes of blood samples were taken from the patients, and they were centrifuged and stored under appropriate conditions in the Health Sciences University, Ankara Dışkapı Yıldırım Beyazıt SUAM, Medical Biochemistry Clinic.

After the 6th month in the treatment of the patients, imaging examinations (chest radiography, CTPA and/or V/P scintigraphy) and TTE, CDU were re-evaluated. MDA level was checked.

Sample Collection and Analysis Methods

Blood samples taken from the selected patients by venipuncture method into the tube containing BD Vacutainer SSTII Advance serum separating gel at the appropriate time were centrifuged at 1500g for 10 minutes and stored at -20°C by placing in capped Eppendorf tubes. On the ELISA working day, the frozen sera were removed from -20°C and allowed to reach room temperature, then vortexed and studied in accordance with the kit insert.

It was studied with ELISA washing device (BioTek ELx50) and ELISA reading device (BioTek Epoch) by using CAYMAN TBARS (product code:10009055) USA 2021 kits.

Statistical Analysis

Existing studies related to the research topic were evaluated and based on these, the minimum number of patients for which statistically significant results could be obtained was found to be 44 when Alpha 0.05 test power was calculated as 0.65 for 80% AUCROC MDA null hypothesis using the "G-Power" program.

Data were analyzed with SPSS version 21.0 (IBM®, Chicago, USA). The conformity of the variables to the normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Shapiro-Wilk test). Normally distributed numerical variables were analyzed with the "T test in Independent Groups" between the two groups, with "ANOVA" between the three groups, and the numerical variables that did not show normal distribution were analyzed with the "Mann Whitney U test" between the two groups and with the "Kruskal Walls" between the three groups. Intragroup analyzes were evaluated using the "Peer t test" in those with normal distribution. Nominal data were evaluated between the two groups using the "Pearson Chi-square test" or the "Fisher's Exact test". The area under the curve (AUC) in the ROC analysis was evaluated to determine the factors that were determinative for the prediction of high-risk PTE, and the data were expressed at a 95% confidence interval. The correlations of normally distributed numerical variables were analyzed with the "Pearson correlation test", and the numerical variables that did not show normal distribution were analyzed with the "Spearman correlation test". In the statistical analyzes of the study, comparisons with a p value below 0.05 were considered statistically significant.

RESULTS

44 patients were included in the study. The mean age of the patients was 54.1±14 years. The male and female gender ratios were equal. At least one acquired risk factor was detected in 75% of the patients. Immobilization and COVID-19 (22.7%) were the most common risk factors. Demographic characteristics of the patients are given in Table 1.

Table 1. Demographic characteristics and risk factors distribution of patients

	All Patients (n=44)	
Age	Mean±SD	54.1±14
Gender	n(%)	
Female		22 (50)
Male		22 (50)
Risk Factors	n(%)	33 (75)
Immobilization		10 (22.7)
COVID-19		10 (22.7)
Obesity		8 (18.6)
History of venous thromboembolism		7 (15.9)
Presence of fracture in the lower extremity		2 (4.5)
Long term travel		2 (4.5)
Major surgical intervention		1 (2.3)
Oral contraceptive usage		1 (2.3)
Congestive heart failure		1 (2.3)

Comorbidity was present in 54.5% of the patients included in the study. The most common comorbidities were diabetes mellitus (18.2%) and hypertension (15.9%). The most common symptoms were Shortness of breath (%79) and chest pain (%28). The distribution of patients according to symptoms is shown in Table 2.

Table 2. Distribution of patients according to symptoms

Symptoms		All Patients (n=44)	<65 Age	≥65 Age	p
Dyspnea *	n(%)	35 (79.5)	26 (78.8)	9 (81.8)	0.829
Chest Pain*	n(%)	28 (63.6)	22 (66.7)	6 (54.5)	0.469
Leg Pain**	n(%)	8 (18.2)	8 (24.2)	0	0.170
Cough**	n(%)	6 (13.6)	4 (12.1)	2 (18.2)	0.630
Palpitation**	n(%)	6 (13.6)	5 (15.2)	1 (9.1)	1.000
Hemoptysis **	n(%)	5 (11.4)	5 (15.2)	0	0.309
Syncope **	n(%)	4 (9.1)	4 (12.1)	0	0.558
Fatigation **	n(%)	4 (9.1)	1 (3)	3 (27.3)	**0.043**

*Chi-square test, **Fisher's exact test

The vital signs of the patients included in the study according to risk groups are given in Table 3.

The mean MDA level at the time of diagnosis was found to be 8.88±3.24 in the low and medium risk group of the patients participating in the study, and 13.06±4.26 in the high-risk group. There was a significant difference between the groups (p=0.003). The results are given in Table 4.

The MDA cut-off value was determined as 11.95 with 80% sensitivity and 62% specificity. MDA levels were found to be predictive for high-risk PTE (AUC: 0.773, 95% CI 0.592-0.953, p=0.017). The ROC curve for high-risk PTE prediction is shown in Figure 1.

While a significant negative correlation was observed between MDA level at the time of diagnosis and saturation a significant positive correlation was observed with ALT, AST and respiratory rate.

Table 3. Distribution of vital clinical and laboratory findings of patients by risk groups

Clinical findings		All patients (n=44)	High risk (n=8)	Medium risk (n=12)	Low risk (n=24)	p value
Systolic blood pressure (mm/Hg)	Median (min-max)	117 (80-185)	89 (80-100)	110 (90-140)	120 (97-185)	A*
Diastolic blood pressure (mm/Hg)	Median (min-max)	70 (50-107)	60 (50-72)	66.5 (60-80)	70 (58-107)	A*
Pulse(beats/min)	Median (min-max)	93 (68-131)	115 (83-131)	97 (78-129)	89.5 (68-110)	A*
Respiratory rate	Median (min-max)	20 (14-35)	24 (18-35)	20 (14-28)	18 (16-24)	A**
Saturation	Median (min-max)	92 (40-98)	82.5 (40-95)	90 (80-95)	94 (83-98)	A**
Laboratory findings						
Ph	Median (min-max)	7.4 (7.18-7.52)	7.39 (6.73-7.48)	7.42 (7.29-7.52)	7.40 (7.33-7.48)	A**
Lactate (mg/dL)	Median (min-max)	1.8 (0.7-17)	3.5 (1-17)	3.15 (0.7-4.6)	1.6 (0.7-2.6)	A*
AST (U/L)	Median (min-max)	20 (10-265)	43 (20-265)	20 (10-33)	17 (10-44)	A*
ALT (U/L)	Median (min-max)	19 (6-192)	49.5 (19-282)	16.5 (6-58)	18.5 (7-58)	A*
Troponin (ng/mL)	Median (min-max)	0.01 (0-3.07)	1.21 (0.78-3.07)	0.36 (0-0.97)	0.01 (0-0.29)	A*
ProBNP (pg/ml)	Median (min-max)	329 (5.97-10852)	2017 (1165-3220)	977 (200.6-10852)	145.5 (5.97-1821)	A*
D-Dimer (µg/mL)	Median (min-max)	4.46 (0.76-25)	7.06 (4.62-25)	4.58 (1.07-25)	2.98 (0.76-7.54)	A*

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, BNP: Brain natriuretic peptide, A: High risk and low risk, *p<0.05; **p≥0.05, Kruskal Wallis test was used.

Table 4. Distribution of malondialdehyde levels by risk groups				
		Low+Medium Risk	C	P
MDA*	Mean±SD	8.88±3.24	13.06±4.26	**0.003**
MDA: Malondialdehit, *Independent group T test				

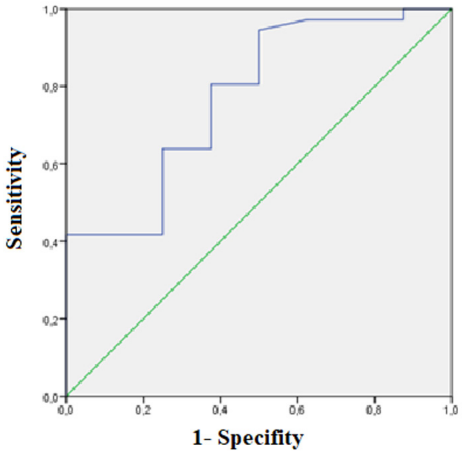

Figure 1: ROC analysis for prediction of high-risk pulmonary thromboembolism

A significant decrease was observed in MDA levels after the treatment of the patients included in the study.

The MDA levels before and after treatment in the patients included in the study are shown in Table 5.

Table 5. Malondialdehyde levels				
MDA level		Pre-treatment	Post-treatment	p value
All patients*	Mean±SD	9.64±3.76	4.74±2.48	**<0.001**
<65 years*	Mean±SD	8.8±3.85	4.2±2.31	**<0.001**
≥65 years*	Mean±SD	8.6±3.66	3.6±3.05	**<0.001**

MDA levels at diagnosis in patients whose thrombus continued after treatment were found to be higher than the other group. (p<0.001). MDA levels examined after treatment were higher in patients with chronic thrombus. (p<0.001).

It was determined that MDA levels measured at the time of diagnosis were not predictive of chronic thromboembolism (AUC:0.605, 95% CI 0.424-0.786, P=0.252).

DISCUSSION

Pulmonary thromboembolism is a life-threatening emergency condition with a high mortality rate despite effective treatment as a result of occlusion of the pulmonary arterial bed. Although the widespread use of BTPA and d-dimer provides some convenience in diagnosis, it makes the diagnosis difficult due to the variability in the clinical picture. In addition, most deaths occur in the first hour in high-risk PTE requiring emergency reperfusion therapy.[9] For this reason, early diagnosis and appropriate treatment are life-saving.

Pulmonary thromboembolism is often considered a disease of advanced age.[10] In a study, the average age was found to be 60.2 years.[11] The mean age of the patients participating in our study was 54.1 years. The low mean age is due to the fact that PTE is more mortal in older patients, they do not continue their follow-up, and they are not included in the study because their comorbidities contribute to oxidative stress.

There is no certainty that sex difference affects the incidence of pulmonary thromboembolism. However, the overall incidence is often thought to be equal for both sexes.[12] In our study, male and female sex ratios were found to be equal, in line with the literature.

Approximately 75% of patients with pulmonary thromboembolism had at least one underlying risk factor.[13] In our study, 75% of the patients had at least one acquired risk factor, in line with the literature. The most frequently reported risk factors in the GARFIELD VTE study were previous operation, recent hospitalization, and lower extremity fracture, respectively.[11] The most common risk factors in our study were COVID-19 and immobilization. This difference in risk factors is due to the fact that our study was conducted under pandemic conditions.

The clinical picture in pulmonary thromboembolism is highly variable. It can take place in a wide spectrum ranging from an asymptomatic picture to sudden death. In the study by Özsu et al.[14], in which they evaluated the national data of PTE, the two most common symptoms were found to be dyspnea and pleuritic chest pain, respectively. In our study, the two most common symptoms were dyspnea and chest, similar to the literature.

It was shown that mortality is higher in PTE patients with high troponin levels.[15] In the study conducted by Lankeit et al.[16], it was stated that the negative predictive value of the high-sensitivity troponin test being normal is 98% in determining the poor prognosis. In our study, troponin levels were found to be significantly lower in the low-risk group in line with the literature.

It was reported that elevated natriuretic peptides are associated with early mortality.[17] Chen et al.[18] stated that NT-proBNP is a very sensitive marker in determining right ventricular dysfunction and mortality, and mortality is significantly reduced if it is detected in low levels. In our study, natriuretic peptide levels were found to be significantly lower in the low-risk group, in line with the literature.

The most toxic one of the compounds formed as a result of lipid peroxidation are aldehydes. As a result of this reaction, cytotoxic and mutagenic MDA is formed. It can easily pass through the cell membrane and react with the structural components of DNA. The high reactivity of MDA has made it one of the most frequently used parameters to measure peroxidation level and oxidative stress. Oxidative stress plays a role in many diseases, including VTE.[19]

It has been shown that increased oxidative stress in ischemic cerebrovascular disease causes cellular damage by uncontrolled increase of inflammatory mediators.[20] In the study by Özkul et al.[21], MDA levels were correlated with clinical outcomes, and an increase in MDA level was associated with more severe disease clinically. Similarly, in our study, high-risk PTE cases were shown to have higher MDA levels compared to other groups, which we think is of vital importance in the early diagnosis of the patient group to be given reperfusion therapy.

In the study by Aykal et al.[22], it was stated that the oxidative balance shifted towards the oxidant side in VTE patients. In a study evaluating MDA in PTE, it was shown that there was a significant increase in MDA level compared to the control group.[23] In the study of Halıcı et al.[24], it was shown that MDA was higher in acute PTE patients compared to the control group, and it still remained high after one month of treatment. In our study, it was shown that the

MDA level in patients with chronic thrombus is high at the time of diagnosis, as well as remained elevated at the end of treatment. As a result of these results, we think that it can be a guide for the evaluation of antioxidant treatments in patients with ongoing thrombus burden.

Pulmonary arterial hypertension (PAH) is a disease of unknown etiology. It has been shown that an increase in ROS levels reduces the bioavailability of nitric oxide, leading to the development of PAH. In the study of Reis et al.[25], it was shown that the increase in MDA level in PAH patients plays a vital role in the progression of pulmonary hypertension. In the study of Smukowska et al.[26], it was shown that MDA levels increase in CTEPH. All these results show that oxidative stress plays an important role in the remodeling of the precapillary pulmonary arteries.

Acute PTE is mostly considered to be a reversible disease, and patients are considered to be completely cured with appropriate anticoagulant therapy. However, in the study of Morris[27], it was reported that more than half of the patients had residual perfusion defects in their control visits 6 months after acute PTE. In our study, it was determined that thrombus persisted in 36.4% of the patients after 6 months of treatment. Since right heart catheterization was not performed in these patients, they could not be evaluated in terms of PH. However, considering that the presence of chronic thrombus is a risk factor for the development of CTEPH, we think that close follow-up is important in patients with continuing MDA elevation.

CONCLUSION

It has been shown that oxidative stress is higher in high-risk PTE compared to other risk groups. MDA can be used as a reliable biomarker in this patient group to avoid delay in treatment. In cases where resolution cannot be achieved completely in acute PTE, the continuation of oxidative stress is an important condition in the remodeling of the precapillary pulmonary artery.

In addition, no other long-term study evaluating the oxidative stress in acute PTE could be seen in the literature. For this reason, we think that our study will shed light on more comprehensive studies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ethical Committe of Dışkapı Yıldırım Beyazıt SUAM Clinical Researches (Date: 16.03.2020, Decision No: 84/16).

Informed Consent: All patients signed and free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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REFERENCES

1. Cohen A, Jeyaindran S, Kim JY, et al. Treating pulmonary embolism in Pacific Asia with direct oral anticoagulants. *Thromb Res*. 2015; 136(2):196-207.
2. Rosendaal FR. Risk factors for venous thrombotic disease. *Thromb Haemost*. 1999;82(2):610-619.
3. 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). *J Am Coll Cardiol*. 2011;57(6):700-706.
4. Nijkeuter M, Hovens MM, Davidson BL, Huisman MV. Resolution of thromboemboli in patients with acute pulmonary embolism: a systematic review. *Chest*. 2006;129(1):192-197.
5. Lang IM, Pesavento R, Bonderman D, Yuan JX. Risk factors and basic mechanisms of chronic thromboembolic pulmonary hypertension: a current understanding. *Eur Respir J*. 2013;41(2):462-468.
6. Karabulut H, Gülay MŞ. Serbest radikaller. *MAKÜ Sağ Bil Enst Derg*. 2016;4(1):50-59.
7. Botham KM, Moyes PA. Fizyolojik öneme sahip lipidler. In: Granner RK, Mayes DK, Rodwell RA, Murray VW, eds. Harper'ın biyokimyası. Barış Kitabevi, 1996.
8. Mühl D, Füredi R, Cristofari J, et al. Evaluation of oxidative stress in the thrombolysis of pulmonary embolism. *J Thromb Thrombolysis*. 2006;22(3):221-228.
9. Tapson VF, Weinberg AS. Overview of management of intermediate- and high-risk pulmonary embolism. *Crit Care Clin*. 2020;36(3):449-463.
10. Heit JA. Epidemiology of venous thromboembolism. *Nat Rev Cardiol*. 2014;12(8):464-474.
11. Ageno W, Haas S, Weitz JI, et al. Characteristics and management of patients with venous thromboembolism: the GARFIELD-VTE registry. *Thromb Haemost*. 2019;119(2):319-327.
12. Yoshikawa Y, Yamashita Y, Morimoto T, et al. Sex differences in clinical characteristics and outcomes of patients with venous thromboembolism - from the COMMAND VTE registry. *Circ J*. 2019;83(7):1581-1589.
13. White RH. The epidemiology of venous thromboembolism. *Circulation*. 2003;107:(1)4-8.
14. Özsu S, Özlü T, Bülbül Y. Ulusal verilerle pulmoner tromboemboli. *Tuberk Toraks*. 2009;57(4):466-482.
15. Becattini C, Vedovati MC, Agnelli G. Prognostic value of troponins in acute pulmonary embolism: a meta-analysis. *Circulation*. 2007; 116(4):427-433.
16. Lankeit M, Jiménez D, Kostrubiec M, et al. Predictive value of the high-sensitivity troponin t assay and the simplified pulmonary embolism severity index in hemodynamically stable patients with acute pulmonary embolism: a prospective validation study. *Circulation*. 2011;124(24):2716-2724.
17. Kucher N, Printzen G, Goldhaber SZ. Prognostic role of brain natriuretic peptide in acute pulmonary embolism. *Circulation*. 2003;107(20):2545-2547.
18. Chen YL, Wright C, Pietropaoli AP, et al. Right ventricular dysfunction is superior and sufficient for risk stratification by a pulmonary embolism response team. *J Thromb Thrombolysis*. 2020;49(1):34-41.
19. Bulkley GB. The role of oxygen free radicals in human disease processes. *Surgery*. 1983;94(3):407-411.
20. Pradeep H, Diya JB, Shashikumar S, Rajanikant GK. Oxidative stress--assassin behind the ischemic stroke. *Folia Neuropathol*. 2012;50(3):219-230.
21. Özkul A, Akyol A, Yenisey C, Arpacı E, Kıylıoğlu N, Tataroğlu C. Oxidative stress in acute ischemic stroke. *J Clin Neurosci*. 2007;14(11):1062-1066.
22. Aykal G, Güven R, Yeğin A, Ellidağ HY, Bayindir A, Yilmaz N. The diagnostic value of oxidative/antioxidative balance parameters in venous thromboembolism. *Clin Lab*. 2015;61(7):769-775.
23. Batmaz E, Edis Çakır E, Eskiocak S, Hatipoğlu ON, Kaya S. The diagnostic value of oxidative stress products in pulmonary embolism. *Turk Thorac J*. 2011;12(3):100-104.
24. Halıcı B, Sarınç Ulaşlı S, Günay E, et al. Assessment of inflammatory biomarkers and oxidative stress in pulmonary thromboembolism: follow-up results. *Inflammation*. 2014;37(4):1186-1190.
25. Reis GS, Augusto VS, Silveira AP, et al. Oxidative-stress biomarkers in patients with pulmonary hypertension. *Pulm Circ*. 2013;3(4):856-861.
26. Smukowska-Gorynia A, Rzymski P, Marcinkowska J, et al. Prognostic value of oxidative stress markers in patients with pulmonary arterial or chronic thromboembolic pulmonary hypertension. *Oxid Med Cell Longev*. 2019;3795320.
27. Morris TA. Why acute pulmonary embolism becomes chronic thromboembolic pulmonary hypertension: clinical and genetic insights. *Curr Opin Pulm Med*. 2013;19(5):422-429.