American Journal of Emergency Medicine 34 (2016) 2315–2319

Contents lists available at ScienceDirect

American Journal of Emergency Medicine

journal homepage: www.elsevier.com/locate/ajem

Original Contribution

The prognostic importance of thiol/disulfide homeostasis in patients with acute pulmonary thromboembolism

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ARTICLE INFO

Article history:
Received 5 May 2016
Received in revised form 8 August 2016
Accepted 20 August 2016

ABSTRACT

Objective: The aim of this study was to evaluate the role of thiol/disulfide homeostasis in acute pulmonary embolism (APE) and investigate its compliance to show hospital mortality of patients with APE.
Material and Methods: A total of 173 participants including 113 patients with APE, and 60 healthy individuals were included in the study. APE group was categorized into two subgroups according to Pulmonary Embolism Severity Index (PESI) clinic risk score (PESI low group [n = 71, class 1-3] and PESI high group [n = 42, class 4-5]).
Results: Mean level of native thiol was lower and disulfide level and disulfide/total thiol ratio were higher in APE group than control group. In APE group, 14 patients died during hospitalization. Native thiol and disulfide level, presence of shock, heart rate, oxygen saturation, right ventricular dysfunction, N-terminal pro–brain natriuretic peptide, and creatinine levels were found to have prognostic significance in univariate analysis. On multilvariable logistic regression analysis, native thiol and disulfide level (odds ratio [OR], 1.16(0.87-1.36); $P = .010$ and OR, 1.49; $P = .015$, respectively), presence of shock (OR, 1.04; $P = .012$) and N-terminal pro–brain natriuretic peptide (OR, 1.67; $P = .002$) were strong predictors for APE-related hospital mortality after the adjustment of other potential confounders.
Conclusion: We have shown that thiol/disulfide homeostasis can be altered during APE and associated with worse hemodynamic parameters, and may be used as a prognostic marker for hospital mortality.

1. Introduction

Acute pulmonary embolism (APE) is a life-threatening and common cardiopulmonary disease with a mortality rate of 15% to 20% [1]. Physicians need to be more careful for the patients with the suspicion of APE because the clinical presentation of APE is often nonspecific and equivocal [2]. Although a lot of markers including cardiac troponin, and brain natriuretic peptide have been studied in the setting of APE and significant reduction in morbidity and mortality of APE has been achieved in recent years, there is still a gap in early diagnosis, risk stratification, and management strategy of APE [3-6].

Oxidative stress occurs as a result of degradation the balance of reactive oxygen species (ROS) and the antioxidant defense system [7]. Thiols are a class of organic compounds that contain a sulfhydryl group (−SH) with in cytosol and mitochondria, and binding particularly in cysteine protein [8]. Cysteine residues in the active sites of proteins detoxify ROS and reduce oxidized protein thiols [9,10]. Under conditions of oxidative stress, oxidation of cysteine residues can result in the reversible formation of disulfide bonds, and present various range of products. These bonds can be reduced again to thiol groups, and homeostasis goes on [11]. This formation (S glutathionylation) can directly alter or regulate protein function (redox regulation) and may as well have a role in protecting from irreversible (terminal) oxidation and cell loss. Thus, thiols contribute the major portion of the total antioxidants level and play an important role in defense against radical oxygen species (ROS) [12].

During excess oxidative conditions, indecent forms of thiol-disulfide accumulation may occur. Increased disulfide level is associated with increased oxidative stress, and often associated with variety of disorders such as diabetes mellitus, atherosclerosis, cancer, and hypertension [13-15]. Although increased oxidative stress is also known to be involved in the prognosis of pulmonary embolism [16], the alteration of thiol/disulfide homeostasis in patients with APE is unknown. Thus, we aimed to investigate the possible role of disulfide/thiol couple in APE patients and aimed to study its usability as a marker for early mortality. To the best of our knowledge no studies up to date investigated thiol/disulfide homeostasis as a novel oxidative stress marker in patients with APE, and compared the results with healthy controls.

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http://dx.doi.org/10.1016/j.ajem.2016.08.039
0735-6757/

2. Material and Methods

This prospective case-control study was conducted at Adana Numune Education and Research hospital, between December 2015 and March 2016. A total of 113 patients who were admitted to our emergency department due to PE related symptoms and diagnosed with APE by multislice spiral computerized tomography according to recommendations of guidelines were enrolled to the current study [17]. Pulmonary Embolism Severity Index (PESI) clinic risk score was calculated by starting with the patient's age and adding additional points for other factors presented as previously and APE patients were categorized into two subgroups according to PESI risk score [18]. Patients with PESI score ≤ 105 (patients in class 1-3) served as PESI low group and patients with PESI score> 106 (patients in class 4-5) served as PESI high group. In addition, 60 age- and gender-matched healthy adults who admitted to hospital for check-up, and did not have an anamnesis of acute or chronic PE or pulmonary hypertension served as controls.

A detailed history was obtained from each patient. Patents with history of diabetes mellitus, coronary artery disease, systemic hypertension, chronic obstructive lung disease, chronic pulmonary hypertension, acute or chronic renal failure, acute or chronic liver disease, presence of neoplastic diseases, active infectious or inflammatory diseases, hematologic disorders, previous stroke, rheumatologic diseases, familial hypercholesterolemia, major depression, recent major surgical procedure, and heart failure were excluded to the current study. Patients taking antioxidant drugs such as b-blocking agents, angiotensin-converting enzyme inhibitors and statins, vitamins, diuretics, hormone replacement therapy, oral contraceptives, and alcohol were also excluded from the study.

The primary efficacy end point was a composite of the rate of death from cardiogenic shock secondary to right ventricular failure during the hospitalization period. The Local Ethics Committee assessed and approved the study and written informed consent for participation in the study was obtained from all individuals.

2.1. Echocardiographic examination

All patients were underwent transthoracic echocardiographic (TTE) examinations using commercially available equipment (Vivid-7; GE Vingmed Sound, Horten, Norway) with a 2.5-3.5 MHz transducer within 48 hours of hospital admission by one experienced cardiologist who was blinded to patients and the study plan. Chamber sizes were defined according to recent guidelines and Left ventricular ejection fraction (LVEF) was measured by bi-plane Simpson's method [19]. Right ventricular (RV) dysfunction was defined as dilatation of the right ventricle (RV dimension >3.4 cm at basal plane or <3.8 cm at midplane), combined with the presence of McConnell sign [20]. Severe tricuspid regurgitation was defined by using color flow jet Doppler signal intensity in combination with vena contracta width according to guideline recommendations and systolic pulmonary artery pressure was calculated from Bernoulli equation as previously demonstrated [21,22].

2.2. Laboratory Analysis

Blood samples were obtained within 6 hours of presentation before starting any medication in APE group, and the samples of the controls were obtained in the morning, after a fasting period of 12 hours. Biochemical measurements were performed within 30 minutes after blood collection and serum samples for thiol were separated after centrifugation at 1500 g for 10 minutes, put into plain tubes and stored at −80 C until analysis.

Biochemical analyses were performed by an automatic blood counter (Roche Diagnostics, Indianapolis, IN, USA) with commercially available kits. The levels of N-terminal pro–brain natriuretic peptide (NT pro-BNP) were assessed by using immunoturbidimetry (Beckmann assay 360, Bera, CA). Hematological parameters were measured from tripotassium ethylenediaminetetraacetic acid-based anticoagulated blood samples and assessed by a Sysmex K-1000 (Block Scientific, Bohemia, NY) autoanalyzer within 30 min of sampling.

Thiol/disulfide homeostasis was measured as described previously [23]. By using sodium borohydrate (NaBH4), disulfide bonds (−S–S–) in the sample were reduced to functional thiol groups. Total thiol (−S−S− + −SH) comprises native and reduced thiol. We used dissolved NaBH4 in 1000 mL water–methanol solution at the same rate. Finally that is a reductant solution (10 μL) for determining total thiol (−S−S− + −SH). Similarly, after dissolved sodium chloride in 1000 mL of water–methanol solution, we obtained reductant solution (10 μL) for determining native thiol (−SH) content. The disulfide parameter is a value which can be calculated automatically as half of the difference between total thiol and native thiol. Native thiol, total thiol and disulfide numerical values were obtained by after oxidation reaction using chloramine-T. After calculation the main parameters, the rate was obtained as disulfide/total thiol (−S−S−)/(−S−S− + −SH). The intra-assay coefficient of variation was evaluated by performing 10 repetitions in a single analytical run, using serum of healthy subjects. The intra-assay coefficient of variation was found 1.7%. The inter-assay coefficient of variation was evaluated in triplicate (on three different dispensing cycles) in five different analytical runs using serum of healthy subjects. The inter-assay coefficient of variation was found 2.0%.

2.3. Statistical analysis

SPSS statistical software (version 16.0, SPSS, Chicago, IL) was used for statistical analysis of data. Continuous variables were expressed as mean ± SD or interquartile range in the presence of abnormal distribution, and categorical variables as percentages. Comparisons between groups of patients were made by use of a χ^2 test for categorical variables, an independent-samples t test for normally distributed continuous variables, and a Mann-Whitney *U* test when the distribution was skewed. The correlations of both native thiol and disulfide levels with other study parameters were evaluated either via Pearson or Spearman correlation tests. We used univariate logistic regression analysis to quantify the association of variables with APE-related in-hospital mortality. Variables found to be statistically significant in univariate analysis and other potential confounders were used in a multivariate logistic regression model with the forward stepwise method in order to determine the independent prognostic factors of early mortality. In the multivariate regression analyses, thiol and disulfide level were evaluated as continuous variables. $P < .05$ was accepted to be statistically significant.

3. Results

A total of 173 participants were enrolled in to the study including 113 patients with APE as the APE group, and 60 healthy subjects as the controls. In APE group, there were 71 patients in PESI low group (PESI score ≤105) and 42 patients in PESI high group (PESI score >106). The baseline demographic, laboratory and echocardiographic characteristics of the APE group (PESI low–PESI high group) and the control group are shown in Table 1.

In laboratory analyses, serum NT pro-BNP levels were found higher in PESI high group than PESI low group and control group ($P = .021$ and $P < .001$; respectively).

In TTE examination, RV diameter, RV inflow velocities, and systolic pulmonary artery pressures were found higher in PESI high group than PESI low group. The signs of hemodynamic impairment (increased heart rate, systemic hypotension) because of RV dysfunction due to overload were worse in PESI high group than PESI low group. Total of 21 patients in PESI high group and 7 patients in PESI low group had cardiogenic shock due to RV dysfunction ($P < .001$) (Table 1).

Mean native thiol levels ($P < .001$), and mean total thiol levels ($P = .001$) were found lower, whereas mean disulfide ($P = .002$), and mean disulfide/total thiol ratios ($P < .001$) were found higher in patients with APE than control. Patients in PESI high group had lowest mean value of native thiol and