RESEARCH ARTICLE

Diagnostic Value of Superoxide Dismutase in Tuberculous and Malignant Pleural Effusions

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Abstract

The aim of this study was to investigate the diagnostic value of superoxide dismutase (SOD) in tuberculous pleural effusions (TPEs) and malignant pleural effusions (MPEs). Pleural effusion (PE) samples from 100 patients were classified on the basis of diagnosis as TPE (n=57) and MPE (n=43). The activity of SOD was determined by pyrolgallol assay. A significant difference was observed in SOD activity ($P<0.01$) between TPE and MPE, levels of being significantly higher in TPE compared to MPE. With a threshold value of 41 U/L, the area under the ROC curve was 0.653, SOD had a sensitivity of 61.4% and a specificity of 61.0% for differential diagnosis. Thus, SOD activity in PE was not a good biomarker in differentiating TPE and MPE. To the best of our knowledge, five SOD isoforms may be present in PE. Identification of which SOD contributes to the difference of SOD level between TPE and MPE is very important for illustrating mechanisms and improving the differential diagnostic value.

Keywords: Superoxide dismutase - tuberculous pleural effusion - malignant pleural effusion - diagnosis

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Introduction

Tuberculosis and malignant diseases are major causes of pleural effusion (PE) (Porcel, 2009; Lombardi et al., 2010). Massive pleural effusion causes severe dyspnea due to progressive respiratory failure, and markedly affects the prognosis of patients with either malignant disease or tuberculosis (TB). Therefore patients with pleural effusion need to be promptly and accurately diagnosed and immediately treated. However, differentiating tuberculous pleural effusion (TPE) from malignant pleural effusion (MPE) can be a critical problem.

In order to achieve a definitive diagnosis of tuberculous pleurisy, Mycobacterium tuberculosis (M.TB) must be isolated from the culture of pleural fluid or tissue, the presence of granulomas in pleural tissue is suggestive (Valdes et al., 2003), the sensitivity of these methods is not sufficiently high even if histological examination of a pleural biopsy specimen and culture of a pleural fluid are combined (Rahman et al., 2004). For diagnosis of MPE, the presence of tumor cells in pleural effusion is a diagnostic marker, but the probability of finding them is low. Accordingly, a reliable clinical marker providing rapid and accurate diagnosis of TPE or MPE is urgently required (Liang et al., 2008).

A lot of studies have dealt with the oxidative stress in tuberculosis and lung cancer (Golubovic et al., 2010; Akca et al., 2012; Zanini et al., 2013). Superoxide dismutase (SOD), an antioxidant enzyme, changes superoxide anion into hydrogen peroxide and oxygen, has been evaluated in these studies. Meanwhile, SOD has clinical implications in monitoring of lung cancer and diagnosis of Alzheimer's disease, radiation pneumonitis and human sepsis (Guerreiro et al., 2010; Carpagnano et al., 2012; Lopez et al., 2012; Wang et al., 2012).

Based on the above problems, the idea was to collect samples of TPE and MPE, determine the level of SOD by colorimetric method, and explore its value in the differential diagnosis between TPE and MPE.

Materials and Methods

Study subjects and sampling

This study has been conducted by the Department of Lab Medicine and Department of Respiratory Medicine, Shandong Provincial Chest Hospital, Jinan, Shandong Province. Our hospital has eight hundred beds and is a referral center for TB treatment in this area. Written informed consent was taken from all the individuals participating in the study, as also an endorsement from the Ethical Committee of the institute.

Between June and September 2012, patients who aged ≥30 were enrolled in this study. The patients were subsequently included if the examinations of PE and/

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or biopsy specimens established a diagnosis of TPE or MPE. TPE was diagnosed by confirming one of the follows: isolation of M.TB from PE or pleural tissue; pleural biopsy revealing granulomatous tissue in the absence of any evidence of other granulomatous diseases; detection of granulomas in pleural tissue with observation of positive response to anti-TB treatment. Malignant PE was diagnosed if the cytology or pleural biopsy specimen revealed underlying malignancy.

Pleural effusions were collected before any treatment was initiated within 24 h after hospitalization. Pleural effusions were centrifuged at 4˚C 1200 r/min for 15 min, and the supernatants were immediately frozen with 500 μl Ep tubes at -80˚C.

Measurement of SOD

The activity of SOD in PE was measured on Advia 2400 Chemistry system (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA) by a commercially available kits (LABCO, Beijing, China) using pyrolgallol assay as described previously by Markland (Marklund et al., 1974).

Statistical analysis

Statistical analysis was carried out using SPSS 17.0 software and MedCalc Version 8.0.1.0. Distributions were assessed by the Kolmogorov-Smirnov test for normality. Data were expressed as mean ± standard deviation (SD) if they had a Gaussian distribution. Otherwise, median values were presented. The statistical difference between the means was calculated using Student's t test if the population distribution was Gaussian. Otherwise, the Mann-Whitney U test was used for determination of statistical difference. Receiver operator characteristic (ROC) analysis was performed to evaluate sensitivity and specificity of SOD, a cut-off point was determined as the value of the parameter that maximized the sum of specificity and sensitivity. Positive and negative predictive values, positive and negative likelihood ratio were also determined. A P value<0.05 was considered statistically significant.

Results

Characteristics of study participants

Pleural effusions were collected from a total of 100 patients with 43 malignant effusions and 57 tuberculous effusions. Table 1 presents the mean age, sex, and underlying diseases and conditions of the evaluated patients. The mean age of all patients was relatively high at 55.1 years, and 69.0% were men. There was no significant difference of age between patients with TPE and MPE, but existed differences in smoking habit. Patients with pulmonary tuberculosis, pulmonary infection were the most dominant in the TPE group, followed by patients with cardiovascular diseases, tuberculous polyserositis, chronic type B hepatitis, diabetes, sarcoidosis and SLE. The mean age of the 43 MPE patients was 58.5 years, and 69.8% of these patients were men. Cardiovascular diseases, pulmonary infection, diabetes were predominant as underlying diseases in these patients. Other underlying diseases and conditions in patients are also included in Table 1.

Table 1. Characteristics of Patients

	Total (100 cases)	TPE (57 cases)	MPE (43 cases)
Age	55.1±14.0	52.6±14.1	58.5±13.3
Sex, # of male	69.0%(69)	68.4%(39)	69.8%(30)
SOD activity in PE (U/L)	44.7±18.4	49.1±19.3	38.6±15.5
Smoking (pack-years)	20.16±28.00	15.22±27.75	27.15±27.17
Underlying diseases and conditions			
Pulmonary tuberculosis	28	28	
Pulmonary infection	30	25	5
Tuberculous polyserositis	3	3	
Chronic type B hepatitis	3	3	
Diabetes	5	2	3
Sarcoidosis	1	1	
SLE	1	1	
Cardiovascular diseases	9	4	5
COPD	2		2
Bone metastasis	2		2
Malignant serous effusion	3		3
Intracranial metastasis	2		2

SLE, systemic lupus erythematosus; COPD, chronic obstructive pulmonary diseases; Data are as presented as the Mean ± SD

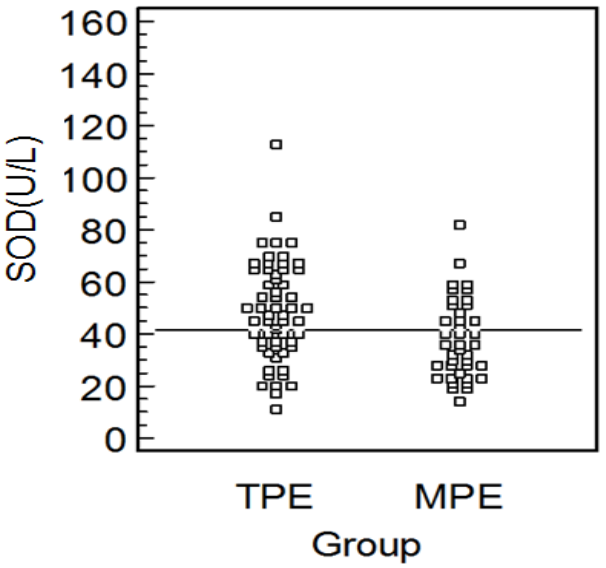

Figure 1. Comparison of SOD Level in TPE and MPE. Horizontal bars indicate cutoff point (41U/L)

Diagnostic value of SOD activity in TPE and MPE

Considering the fact that activities of SOD in TPE and MPE were under Gaussian distribution, results are stated as mean ± SD and presented in Table 1. Student's t test was used to compare levels of SOD activity in TPE and MPE. It showed that statistically significant differences between the two groups (p<0.05, Fiure 1). The activity of SOD in pleural effusions of TPE patients was higher than those of MPE patients.

ROC curve was constructed from SOD assay, comparing TPE patients with MPE subjects. The area under the curve was 0.653 (Figure 2). The selection of the best cutoff point value was based on the level at which the accuracy was maximum. The best cutoff point was found to be 41U/L, with a sensitivity of 61.4% and a specificity of 61.0%. The positive predictive value, negative predictive

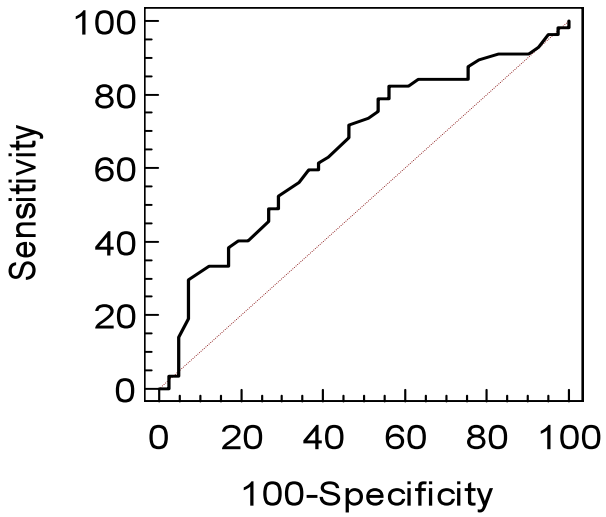

Figure 1. ROC Curve for SOD Assay. The area under the curve was 0.653

value, positive likelihood ratio and negative likelihood ratio of the assay were 86.7%, 83.3%, 1.57 and 0.63, respectively.

Discussion

In the present study, the activities of SOD in TPE were significantly different from in MPE. Comparing with SOD activities in MPE, the SOD activities in TPE were higher. In order to evaluate the role of SOD activity in differentiating TPE and MPE, ROC curve was constructed, and the best cutoff was found to be 41U/L, with the sensitivity and specificity of 61.4%, 61.0% respectively.

Although it showed that pleural SOD activity between TPE and MPE existed statistically significant differences. SOD activity in PE wasn't a good biomarker in differentiating TPE and MPE. To the best of our knowledge, five SOD isoforms may present in PE. Three isoforms of SOD which are from human exist with varying structures, regulation, localizations, and functions (Johnson et al., 2005).Cu, Zn-SOD (SOD1) is typically cytosolic and Mn-SOD (SOD2) is mitochondrial, while extracellular SOD (SOD3) scavenges superoxide radicals in extracellular fluids and spaces (Fattman et al., 2003; Nozik-Grayck et al., 2005). M.TB has two superoxide dismutase proteins, SodC and SodA. SodC is a Cu, Zn superoxide dismutase responsible for only a minor portion of the superoxide dismutase activity of M. tuberculosis (Piddington et al., 2001). SodA is an iron-cofactored enzyme and a virulence factor secreted in large quantities by pathogenic M.TB. SodA can elicit strong immune responses in tuberculosis-infected mice and reducing SodA production by M.TB can enhance rapid mononuclear cell infiltration into the lung and apoptosis of host cells (Andersen, 1994; Edwards et al., 2001). Which isoforms of SOD were responsible for the difference in SOD activity between TPE and MPE? Once the question was solved, it may improve diagnostic value of SOD activity in differentiating TPE and MPE.

Considering the fact that SOD3 and SodA are secreted in large quantities by human and M.TB, respectively, SOD3 and SodA may contribute to increase SOD activity in TPE. SOD3 accounted for 70% of total SOD activity in serum, SOD1 was widely distributed and comprised 90% of the total SOD (Oury et al., 1996; Noor et al., 2002). It was reported that the concentration of SOD1 in TPE (median: 117.7 ng/ml, interquartile range: 75.55-180.7 ng/ml) was close to in MPE (median: 125.3 ng/ml, interquartile range:71.95-201.1 ng/ml). Meanwhile, as a virulent factor is necessary for TB surviving in host, SodA is the only secreted SOD of M.TB. SOD3, or SodA, or both of them may lead to increase of total SOD activity in TPE. To identify which SOD contribute to increasing SOD level is very important for illustrating the mechanism for TPE or MPE and is very helpful to improve diagnostic value of SOD activity in differentiating TPE and MPE.

The SOD activity in serum from patients with lung cancers was markedly lower than the TB patients and healthy control ($p<0.001$), activities of serum SOD in TB patients significantly decreased as compared to healthy controls ($p<0.05$) (Yıldız Güney, 2004). In the present study, we found that pleural SOD activity of TPE was higher than of MPE. SOD is an important antioxidant enzyme catalyzing conversion reaction of superoxide radical to hydrogen peroxide and molecular oxygen. In several studies, decreased serum SOD activities were reported in cancer patients (Kwee et al., 1991; Bhuvarahamurthy et al., 1996). It was reported that human tumor cells shown to produce large amounts of hydrogen peroxide (Szatrowski et al., 1991). hydrogen peroxide, as a major reactive oxygen species (ROS) can cause DNA lesions in malignant cells, which may contribute to metastatic potential of tumors (Jaruga et al., 1994). Oxidative stress plays an important role in the pathogenesis of lung infection, cellular oxidative damage is the result of an imbalance between ROS production and antioxidant cellular defenses. In order to avoid oxidative damage, SOD serves as a protective factor (Hosakote et al., 2009; Rodriguez et al., 2009; Andrades et al., 2011). These imply that low level of SOD in MPE promotes tumor metastasis, otherwise high level of SOD in TPE served as a protective factor.

In fact, 113 TPEs were collected and analyzed, our data indicated that age was significantly and negatively correlated with pleural SOD activity in TPE ($P<0.01$). In order to keep balance between two groups, patients who aged ≤30 years old were excluded. In the present study, there was no significant difference between TPE and MPE in age. The limitation of the study was that statistically difference existed between TPE and MPE in smoking habit. Tobacco smoke exposure affected the balance between oxidative stress and antioxidant capacity of lungs. Preventing environmental tobacco smoke exposure can decrease oxidative damage by increasing levels of 8-OHdG and SOD levels (Doruk et al., 2011). If more patients were enrolled in the study, it may be avoided. Although we tried our best to balance the two groups, we couldn't avoid factors which affected the level of pleural SOD activity completely. In order to evaluate oxidative stress in PE, it is emergent to investigate which factors can affect level of SOD activity in PE, such as, sex, age, ethnicity, lifestyle, air quality and smoking.

In summary, we showed lower pleural SOD activity

in MPE as compared to in TPE. Our data indicated that pleural SOD activity had poor diagnostic value in differentiating TPE and MPE. In order to improve diagnostic value of SOD activity and illustrating the role of SOD in PE, it's need to identify which SOD play important role in increasing total SOD level in TPE.

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