

# Role of Apo E and superoxide dismutase in patients with obstructive lung diseases

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(Submitted: 09 December 2017 – Revised version received: 11 January 2017 – Accepted: 16 February 2017 – Published online: 26 March 2017)

**Objective** Obstructive lung diseases (OLD) are chronic inflammatory disorders of the respiratory tract including asthma and chronic obstructive pulmonary disease (COPD). Apo lipoprotein E (Apo E), is a multifunctional protein as it intervenes the binding of lipoproteins or lipid complexes to specific cell-surface receptors. Experimental studies referred to the function of Apo E as an endogenous negative regulator of airway hyper responsiveness and goblet cell hyperplasia. The protective role of Apo E pathways primarily in respiratory disease was explained in human studies and research utilizing experimental murine model systems. Literature data reveal a strong association between redox status, including the enzyme superoxide dismutase (SOD) with both the development a severity of OLD. This study aims to investigate the relation between SOD antioxidant enzyme activity in addition to investigating the level of Apo E and the development of obstructive lung diseases (OLD).

**Methods** Patients with OLD ( $n = 40$ ) and 40 age-matched healthy controls were enrolled in this study. Serum samples were collected to test the role of Apo E and to test the effect of antioxidant enzyme SOD, and their influence on OLD, all measured by ELISA.

**Result** The results showed a significant decrease in the level of serum SOD activity in patients with OLD when compared with control group ( $P < 0.05$ ). However, the levels of Apo E did not show a significant difference between the two groups.

**Conclusion** Decreased level of antioxidant SOD suggests the presence of an oxidative stress in asthmatic airways favoring a more oxidative state is present in the airway inflammation.

The level of Apo E was non significantly increased in serum of patient, this suggests that protein level of Apo E does not change but may be Apo E gene expression is altered.

**Keywords** apo E, superoxide dismutase, obstructive lung diseases

## Introduction

Chronic respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) are common problems and are regarded as the major public health burdens.<sup>1,2</sup> They are characterized by reversible and irreversible airway obstruction, respectively. Although remain the primary reason of these diseases unknown, progressive and permanent pulmonary tissue damage (airway remodeling) that leads to the total loss of lung function as a result of inflammation which is considered a central feature for them. In fact, the inflammatory mechanisms and other biological pathways involved in asthma and COPD pathogenesis must be explained, in order to find new possible diagnostic/prognostic biomarkers and for the validation of new drug targets.<sup>3</sup>

There are significant differences in the patterns of fundamental inflammation, in spite of the similarity in clinical symptoms of both diseases, they are caused by airway narrowing as a result of inflammation.<sup>4</sup>

In COPD, the inflammatory cell infiltrate in small airways include mainly neutrophils and cytotoxic T cells (cluster of differentiation-8 (CD8) positive lymphocytes). Parenchymal destruction in COPD is associated with loss of lung tissue elasticity, and small airways collapse during exhalation. Asthma has been characterized mainly by Type 2 helper T cell (Th2) cytokine-mediated eosinophilic airway inflammation associated with airway hyperresponsiveness.<sup>2</sup>

Recent studies suggest that 13%–20% of patients with COPD have an overlap phenotype with asthma; this is as high as 50% in patients over 50 years, because of 30% of people with

asthma smoke, a proportion of these will develop chronic air-flow limitation, which is likely to be indistinguishable from COPD.<sup>5–7</sup>

Antioxidants are one of chemical substances can inhibit the oxidation of a molecule. In the living organisms, antioxidants can nullify the pathology effects of oxidation caused by free radicals.<sup>8</sup>

### Superoxide Dismutase

Superoxide dismutase is considered one of oxidoreductases, which catalyze the dismutation of  $O_2^{\cdot-}$  into oxygen and  $H_2O_2$ , therefore it is classified as a major cellular defense against  $O_2^{\cdot-}$  and peroxynitrite.

Thus, Cu or Mn will be important modulator of SOD activity of SOD1/SOD3 or SOD2, respectively.<sup>9</sup>

### Apo Lipoproteine E

Apo lipoproteine E (Apo E) is a protein presents in the interstitial fluid and lymph, as well as in the plasma and is synthesized and secreted from a variety of tissues and different kinds of cells.<sup>10–12</sup> It has three major isoforms (Apo E2, Apo E3, and Apo E4) with different effects on lipid and neuronal homeostasis.<sup>13</sup>

Intracellular Apo E may regulate various cellular processes physiologically or pathophysiologically. Apo lipoprotein (Apo) E is a multifunctional protein as it intervenes the binding of lipoproteins or lipid complexes in the plasma or interstitial fluids to specific cell-surface receptors as a major function of Apo E.<sup>13</sup>

Many respiratory diseases, including asthma, acute lung injury, cancer, emphysema, pulmonary fibrosis, and pulmonary hypertension are associated with Apo E which is expressed by lung cells, which allows Apo E/ low density lipoprotein receptor (LDLR) dependent pathways to modulate normal lung health, as well as the pathogenesis of these diseases. The protective roles of Apo E and Apo lipoproteine A-I (Apo A-I) pathways primarily in lung biology and respiratory disease were explained in human studies and research utilizing experimental murine model systems.<sup>14</sup>

## Materials and Methods

### Assay Procedure

Serum levels of superoxide dismutase were determined by classic Competitive-ELISA using ELISA minikits (Elabscience, China). Whereas serum levels of Apo E were determined by classic Sandwich-ELISA using ELISA minikits (Elabscience, China) according to the instructions enclosed with the kits.

### Calculation of Results

Average the duplicate readings for each standard and sample. Create a standard curve by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis and draw a best-fit curve through the points on the graph. By using professional Microsoft Excel to do this calculation, a best fitting equation of standard curve will be calculated using OD values and concentrations of standard sample. The software will calculate the concentration of samples after entering the OD value of samples.

## Results

The antioxidant status which represented in the present study was reduced SOD which is the master antioxidant in the body. This was achieved by evaluating the changes according to the activity of the disease. A brief illustration in (Table 1) which summarize the overall findings regarding the studied parameters of the current study.

### Superoxide dismutase

The results in Fig. 1 show a significant decrease ( $P < 0.05$ ) in SOD concentration in serum of patient with OLD group compared to the control group.

The data show that the mean  $\pm$  SD of SOD in patients with OLD and control group were  $445.702 \pm 148.217$  and  $520.757 \pm 179.902$  Pg/ml, respectively.

Table 1. Summarizes parameters studied represented by serum (SOD and Apo E) for control and patients with OLD

Parameters	Group	Mean $\pm$ SD	P-value
SOD; pg/ml	Patient	$445.702 \pm 148.217$	< 0.05
	Control	$520.757 \pm 179.902$	
Apo E; ng/ml	Patient	$727.47 \pm 189.31$	> 0.05
	Control	$697.51 \pm 207.32$	

SOD, superoxide dismutase; Apo E, apo lipoprotein E; OLD, obstructive lung disease; Student's *t*-test *P*-value of < 0.05 was considered to be statistically significant.

### Apo Lipoproteine E

The results in Fig. 2 showed non-significant change ( $P > 0.05$ ) in serum Apo E concentration in patients with OLD group compared to the control group. The data show that the mean  $\pm$  SD of Apo E in OLD and control group were  $727.47 \pm 189.31$  and  $697.51 \pm 207.32$  Pg/ml, respectively.

## Discussion

The results of the present study revealed a significant decrease in SOD levels in patients with OLD. An enzymatic antioxidant system is critical for the redox status homeostasis and for protecting human cells. In fact, when this homeostasis is disrupted and there are more reactive species than antioxidants several diseases can develop.<sup>15</sup>

Recent studies indicate that an increased oxidative stress is related to the pathogenesis of COPD,<sup>1,16-18</sup> and contribute this to the fact that COPD patients produce more  $H_2O_2$  compared to "healthy".<sup>19</sup> Another study hypothesized that loss of asthmatic SOD activity is due to greater susceptibility to oxidative inactivation.<sup>20</sup>

An increase in ROS generation in asthma and/or COPD patients is confirmed by the changes in the antioxidant

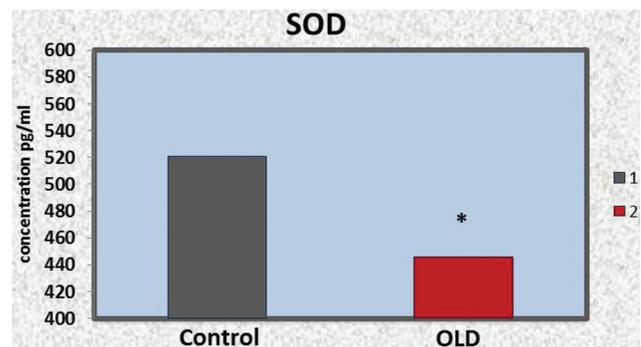


Fig. 1 Serum superoxide dismutase (SOD) activity in patients with obstructive lung disease (OLD) and control. Serum samples were isolated from the blood of patients with OLD. SOD level was assessed by ELISA. Data are expressed as means  $\pm$  SD, for 40 patients, with duplicate measurements. \* indicates significant differences compared to the control  $n = 40$ , (Student's *t*-test,  $P < 0.05$ ).

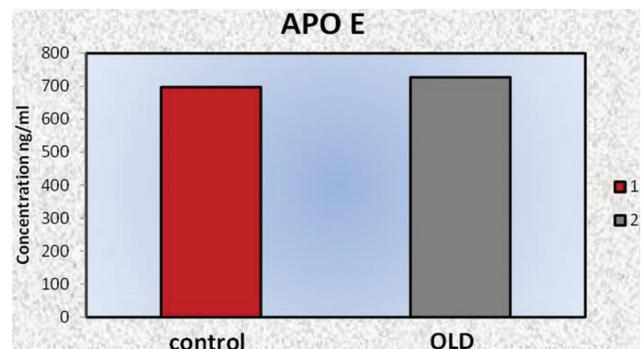


Fig. 2 Serum apo lipoprotein E (Apo E) levels in patients with obstructive lung disease (OLD) and control. Serum samples were isolated from the blood of patients with OLD. Apo E level was assessed by ELISA. Data are expressed as means  $\pm$  SD, for 40 patients, with duplicate measurements, compared to the control group  $n = 40$ , (Student's *t*-test,  $P > 0.05$ ).

enzymes activity. These results support the hypothesis that an oxidant-antioxidant imbalance, associated with oxidative stress in COPD patients, plays an important role in the progression of disease severity.<sup>21</sup>

Previous studies also observed the same fact and concluded that oxidative stress remains increased among asthmatics with low levels of antioxidant activity.<sup>22</sup>

There is a strong evidence that an imbalance between the reducing and oxidizing systems favoring a more oxidative state is present in the airway inflammation and a deficiency in the amount of antioxidants exists in the asthmatic airway.<sup>23</sup>

Superoxide dismutases (SODs) are the major antioxidant defense systems against O<sub>2</sub> radical.<sup>24</sup>

Vulnerability of CuZnSOD influenced by redox likely amplifies injury and inflammation during acute asthma attacks when reactive oxygen species are explosively generated. Overall, this study identifies a new paradigm for understanding the chemical basis of inflammation.<sup>20</sup>

The difference in mean SOD level was found to significantly decrease among asthmatic patients.<sup>25</sup>

Antioxidants not only protect against the direct injurious effects of oxidants, but also alter the inflammatory events that play an important role in the pathogenesis of COPD.<sup>26</sup> The outcome of the present investigation confirms the role of an oxidant-antioxidant imbalance in the etiology of COPD and/or asthma. The data from the current study have shown non-significant increase in the Apo E levels in patients when compared with control group (Fig. 2). This finding is consistent with the results of many previous studies as shown below.

Although the primary function of Apo E is to facilitate lipid transport into cells by receptor-mediated endocytosis

mediated by the LDL receptor, it has been recognized that Apo E modulates a variety of additional important biological functions. Recent studies suggested that Apo E may be involved in therapy strategy for OLD.

Experiments utilizing humanized Apo E knock-in mice have demonstrated that human Apo Eε3 had a protective effect on the severity of HDM-induced airway disease.<sup>27</sup>

The ability of Apo E, which is expressed by lung macrophages, to attenuate AHR, and goblet cell hyperplasia is mediated by low density lipoprotein (LDL) receptors expressed by airway epithelial cells. So, the administration of an Apo E mimetic peptide, corresponding to amino acids 130–149 of the LDL receptor-binding domain of the holo-Apo E protein, significantly reduced AHR and goblet cell hyperplasia in HDM-challenged Apo E<sup>-/-</sup> mice.

These findings identified the Apo E-LDL receptor pathway as a new drug able target for asthma that can be activated by administration of Apo E-mimetic peptides.<sup>28</sup>

## Recommendation

Further studies are to be conducted to predict the causal association of other markers such as lipid profile, Apo A and non-enzymatic antioxidants with the development of OLD; asthma and COPD.

It is worthy to investigate Apo E in cell culture and study the different effect of polymorphic Apo E alleles by using gene expression.

## Conflicts of interest

None. ■

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