Original paper

Role of IL-4 and Glutathione Peroxidase in Patients with Obstructive Lung Diseases

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Abstract

Background: By definition, the obstructive lung diseases, abbreviated as (OLD), are chronic inflammatory conditions of the lungs. These include two distinguished intities, asthma and chronic obstructive pulmonary disease (COPD). In both asthma and COPD the chronic inflammation and structural changes of the respiratory tract are organized by cytokines which have become important targets for the development of new therapeutic strategies in these diseases. Glutathione peroxidases (GPX) is one of the scavengers of antioxidant enzymes against activated oxygen species which is the first discovered in mammals. The efficacy of glutathione peroxidase enzyme is correlated with an amino-acid called selenocysteine especially when found at a position within the catalytic site.
Aim: The goal of the current study is to Predict if there is any association between GPX antioxidant enzyme activities as a marker of oxidative stress status. Furthermore, to test the role of serum level of IL4 as a causative cytokine in the pathway of OLD pathogenesis.
Methods: A total of fourty Patients diagnosed by a specialist physician to have OLD were selected as a test group. In addition, 40 age-and gender- matched apparently healthy persons were invited to participate in the current study after obtaining their consent. Blood samples were processed to measure serum level of IL-4 by ELISA. Similarly, the serum level of enzyme GPX was estimated. Statistical analysis was performed to investigate the association of IL-4 and antioxidant enzyme GPX with the development of OLD.
Result: Data from the current study revealed a significant increase in serum level of the level of IL-4 in the OLD patients in comparison with healthy persons *(P* <0.05). On the other hand, the enzyme serum level of GPX revealed non-significant difference among the study participants.
Discussion: As shown by present results, there is significantly increase in levels of IL-4 in serum of patient group compared to control group. This finding is in agreement with a report which indicated that IL-4 marker of allergic airway inflammation.
Conclusion: Serum levels of inflammatory cytokines IL-4 is associated with the diseases severity of OLD and can be regarded as an important immunological player in the development of this condition.
Keywords: Interleukine-4, Glutathione peroxidase, Obstructive Lung Diseases.

Introduction

Obstructive lung diseases (OLD) such as asthma and chronic obstructive pulmonary disease (COPD) are characterized by reversible and irreversible airway obstruction, respectively. In fact, the inflammatory mechanisms and other biological pathways involved in asthma and COPD pathogenesis must be explained, in order to find new possible

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diagnostic/prognostic biomarkers and for the validation of new drug targets.[1]

Globally, chronic obstructive pulmonary disease (COPD) is one of the most important causes of death affect millions of people worldwide [2] and is regarded as the third leading cause of mortality. Furthermore, COPD is characterized by loss of lung function and by progressive airflow limitation. [3] Signs and symptoms of COPD characterized by cough, dyspnoea and sputum production, or unusually recurrent attacks of common cold which is important for clinical diagnosis. [4]

Asthma is another type of chronic obstructive lung diseases. Asthma is regarded as an obstructive lung condition and being reversible. In addition to chronic inflammatory process in the respiratory airways. In addition to airway hyperresponsiveness (AHR), asthma is correlated with several leading signs such as increassed serum level of total Immunoglobulin E (IgE). [5,6] These features act together with genetic predisposition to cause the eventual development symptoms and signs of asthma including shortness of breath, chronic cough and tightness in the chest. [7]

Antioxidants are chemical substances that can inhibit the oxidation of a molecule. In the living organisms, antioxidants can nullify the pathology effects of oxidation caused by free radicals. [8]

One of an antioxidant enzyme is Glutathione peroxidases -1(GPX-1) that is expressed in most cell types by using electrons provided by glutathione (GSH) as a reduced state. These electrones are used by GTX enzyme to cause reduction of H2O2 (hydrogen peroxide) or other reactive oxygen compounds such as lipid peroxides.[9] It was started that GPX-1 enzyme utilizes a co-enzyme, GSH in the reduction reaction of (H_2O_2). This will result in oxidized glutathione formation. The latter will undergo reduction to be convrted into GSH by the enzyme, glutathione reductase (GR). Inflammation of the lung can be induced by certain reactive oxygen species (ROS) such as $O_2^{\bullet-}$, $ONOO^-$, (H_2O_2), and •OH. They can, also, induce damage to the DNA molecule and denaturate some proteins, cause peroxidation of lipids and even cause pulmonary emphysema. [10]

In both asthma and COPD the chronic inflammation and structural changes of the respiratory tract are organized by cytokines which have become important targets for the development of new therapeutic strategies in these diseases. [11]

Cytokines are means of cellular communication. They are secreted by some immune cells and, specifically, act on certain other cells. [12] At 1990s the first discovery about the critical role ofIL-4 in the development of allergic airway inflammation was begin. [13]

Therefore, in early clinical experiments, the antagonism of IL-4 and IL-13 as a therapeutic trial succeeded in aliveating symptoms of some obstructive lung diseases. The latter findings rise awareness toward the idea of blocking the effect of IL-4 and IL-13 is a rich area of research at the level of clinical trials in treating obstructive and allergic lung disorders. [14]

The main objective of the current study is to declare the hypothesis that IL-4 and Glutathione peroxidase are associated with the development of OLD.

Materials and Methods

The design of this observational study is a cross sectional style. It was performed during a period from April, 2016 through August, 2016 in Al-Aabid primary health care center in Kerbala, Iraq.

Oral and written informed consents were obtained from all patients and control healthy participants in this study.

Criteria for participants' selection

Forty doctor-diagnosed patients with OLD; chronic obstructive pulmonary disease and asthma; 9 men and 31 women, (19-65 years old) have taken part in this study and they were included as the test group. Another

forty persons attending as apparently healthy, age ranged between (19-65 years old) were chosen as non-COPD or asthma disease as control group 11 males and 29 females after taking the agreement .

Patients with doctor-diagnosed OLD who especially those smoking (or not), the period of their disease, treatment and occupation. Frequency and severity of dyspnea attacks together with some other relevant information were recorded by the patients according to a predefined sheet.

Exclusion criteria

Patients with other conditions such as heart diseases, hypertension, diabetes mellitus and those with dyslipidemia were excluded from the study.

Sample collection and processing

After an informed consent was taken, 3-ml venous blood samples were taken from all selected participants under aseptic condition and blood kept in a serum tube. After clotting, samples were centrifuged at 3000 xg for ten to fifteen minutes. Then, sera were stored at -20˚C until use. The sandwich ELISA technique was performed to determine serum level of GPX and IL4 by using ELISA minikits (Elabscience, China). The procedure was done following the kits' instructions[15].

Results

To predict the association among GPX enzyme, IL-4 and the development of (OLD).

The results in figure (1) showed no significant change (P>0.05) in GPX concentration in serum of patient with OLD in comparison with the healthy controls. In addition, the data show that the mean ± SD of GPX in OLD and control group were 749.206 ± 57.63 and 740.479± 84.427 Pg/ml, respectively.

Regarding the IL-4 which is one of an important inflammatory cytokine in patients with OLD, the results in figure (2) reveal that its level is significantly increased in patients with OLD group compared to the control, (P value <0.05). The data show that the mean ± SD of IL-4 in OLD and control group were 296.76 ±247.66 and 182.89 ± 219.418 Pg/ml, respectively.

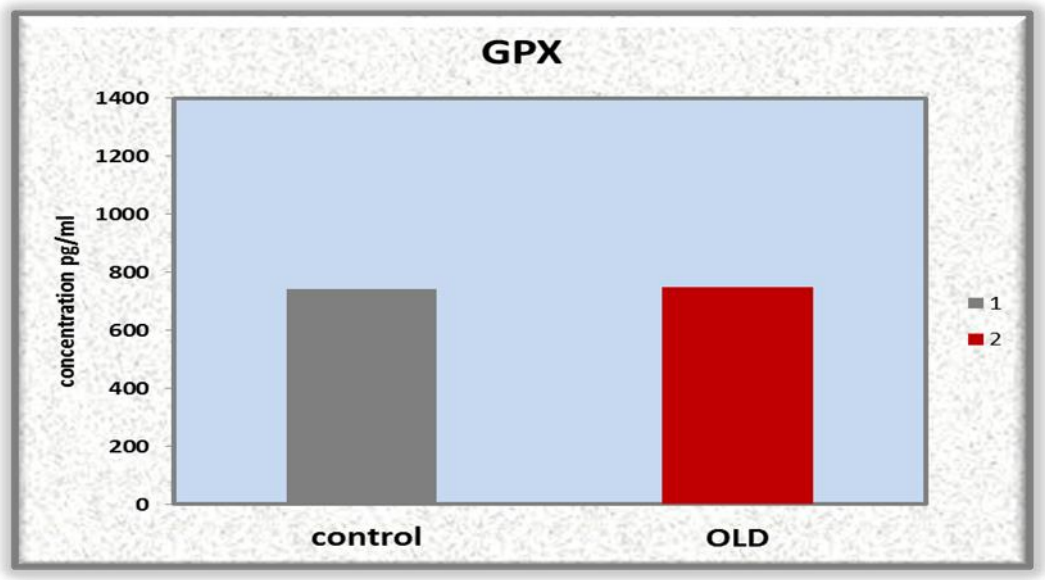

Figure 1. Serum GPX activity in patients with OLD and control. Serum samples were isolated from the blood of patients with OLD. GPX levels was assessed by ELISA. Data are expressed as means ± SD, for 40 patients, with duplicate

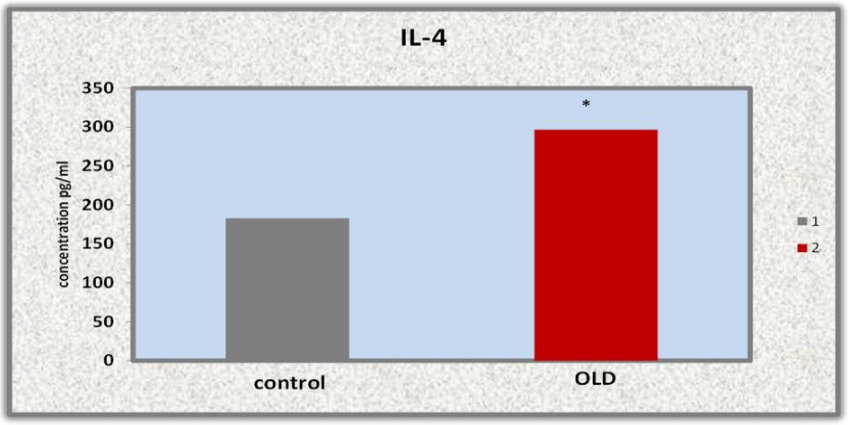

Figure 2. Serum IL-4 levels in patients with OLD and control. Serum samples were isolated from the blood of patients with OLD. IL-4 levels was assessed by ELISA. Data are expressed as means ± SD, for 40 patients, with duplicate measurements. *indicates significant differences compared to the control, n = 40, (Student's t-test, $P < 0.05$).

Discussion

The results in present study also observed no significant difference was found between the patients with OLD and control group. This finding is in agreement with a study which referred no significant differences were shown regarding GPX, GR, and activities between OLD and control groups,[16] and in all study participants (OLD patients, control smokers, control nonsmokers). [17] A study by Biljak [18], demonstrated that Glutathione reductase activity was increased, while GPX activity was decreased in the patients with COPD, when compared to healthy controls, and no significant difference was found between the individual for Obstructive Lung Disease (OLD) stages, most probably because patients selected for the study were in the stable form of the COPD.[18]

As shown by present results, there is significantly increase in levels of IL-4 in serum of patient group compared to control group. This finding is in agreement with a report by Lu [19] which indicated that IL-4 marker of allergic airway inflammation. [19] Expression level of IL-4 was also significantly increased in asthmatic patients and highly correlated within individual subjects [20].

Studies of IL-4 have revealed a wealth of information on the diverse roles of this cytokine in homeostatic regulation and disease pathogenesis. [21,22] Interleukin (IL)-4, also known as B-cell-stimulating factor, is a pleiotropic cytokine. It mainly promotes the proliferation of T cells and induces antibody production by B cells, and increases the recruitment of inflammatory cells. [23,24], this associated with oxidative stress mediated damage.

Conclusion

Serum levels of inflammatory cytokines IL-4 are related with severity of airway diseases and could be potential markers for the evaluation of OLD.

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