

## Original paper

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

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## Abstract

**Background:** Obstructive lung diseases (OLD) are chronic inflammatory disorders of the respiratory tract including 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. Their activity depends on the presence of the rare amino-acid residue selenocysteine at the catalytic site.

**Aim:** This study aims to investigate the relation between GPX antioxidant enzyme activities as a marker of oxidative stress status in addition to investigating the level of IL-4 with 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 IL-4 and to test the effect of antioxidant enzyme GPX, and their influence on OLD, all measured by ELISA.

**Result:** The results showed a significant increase in the level of serum IL-4 activity in patient with OLD when compared with control group ( $P < 0.05$ ). However, the levels of GPX did not show a significant difference between the two groups.

**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 are related with severity of airway diseases and could be potential markers for the evaluation of OLD.

**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

diagnostic/prognostic biomarkers and for the validation of new drug targets.<sup>(1)</sup>

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of death affect millions of people worldwide<sup>(2)</sup> and is currently the third largest cause of death in the world. COPD is characterized by loss of lung function and by progressive airflow limitation.<sup>(3)</sup> Signs and symptoms of COPD characterized by shortness of breath, a chronic cough, sputum production, or frequent winter colds and a

history of exposure to risk factors for the disease which is an important for clinical diagnosis.<sup>(4)</sup>

Asthma is another type of chronic obstructive lung diseases characterized by reversible airway obstruction and chronic airway inflammation and is associated with a number of intermediate phenotypes such as elevation of the total serum Immunoglobulin E (IgE) and airway hyper-responsiveness (AHR).<sup>(5,6)</sup> In genetically susceptible individuals, these interactions can lead the patient with asthma to symptoms of breathlessness, wheezing, cough, and chest tightness.<sup>(7)</sup>

Antioxidants are one of 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.<sup>(8)</sup>

One of an antioxidant enzyme is Glutathione peroxidases -1(GPX-1) that is expressed in most cell types by using electrons provided by reduced glutathione (GSH) to reduce hydrogen peroxide or lipid peroxides.<sup>(9)</sup> GPX-1 uses GSH as a cofactor to reduce ( $H_2O_2$ ), resulting in the formation of oxidized glutathione, which can then be reduced to GSH by glutathione reductase (GR). The ROS  $O_2^{\bullet-}$ ,  $ONOO^-$ , ( $H_2O_2$ ), and  $\bullet OH$  can then cause lung inflammation, DNA damage, protein denaturation, lipid peroxidation, and emphysema.<sup>(10)</sup>

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.<sup>(11)</sup>

Cytokines are released by cells have a specific effect on the interactions and communications between cells.<sup>(12)</sup> At 1990s the first discovery about the critical role of IL-4 in the development of allergic airway inflammation was begin.<sup>(13)</sup>

Therefore, in early clinical experiments the simultaneous blockage of IL-4 and IL-13 has successfully mitigated symptoms of

allergic asthma and other. This review brings to light new data demonstrating that agents targeting IL-4 and IL-13 are proportionately secure and effective medications in blocking the inflammatory cascade responsible for allergic asthma and other disease.<sup>(14)</sup>

The aim of this study is to declare the role of IL-4 and Glutathione peroxidase in patients with obstructive lung diseases (OLD) and it is hypothesis is that these markers contribute to the development of OLD.

## Materials and Methods

This is a cross sectional observation study 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 by vein puncture and pushed slowly into plain disposable tubes. Blood was allowed to clot at 37°C for 10-15 minutes and then centrifuged at 3000 x g for approximately 10-15 minutes then the sera were obtained and stored at -20°C until use for ELISA determination of antioxidant enzyme; GPX, and IL4 serum levels which were determined by classic sandwich -ELISA using ELISA minikits (Elabscience, China) according to the instructions enclosed with the kits. The sandwich technique is used to identify a specific sample antigen. The well surface is prepared with a known quantity of bound antibody to capture the desired antigen. After nonspecific binding sites are blocked using bovine serum albumin, the antigen-containing sample is applied to the plate. A specific antibody is then added that “sandwiches” the antigen. Enzyme-linked secondary antibodies are applied that bind to the antigen. Unbound antibody–enzyme conjugates are washed off. Substrate is added and is

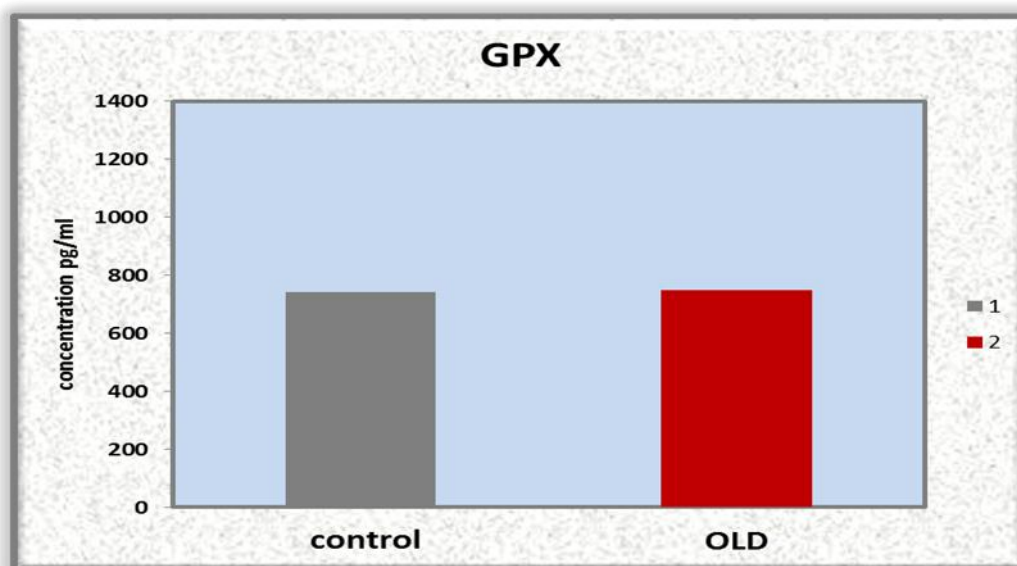
enzymatically converted to a color that can be later quantified <sup>(15)</sup>.

## Results

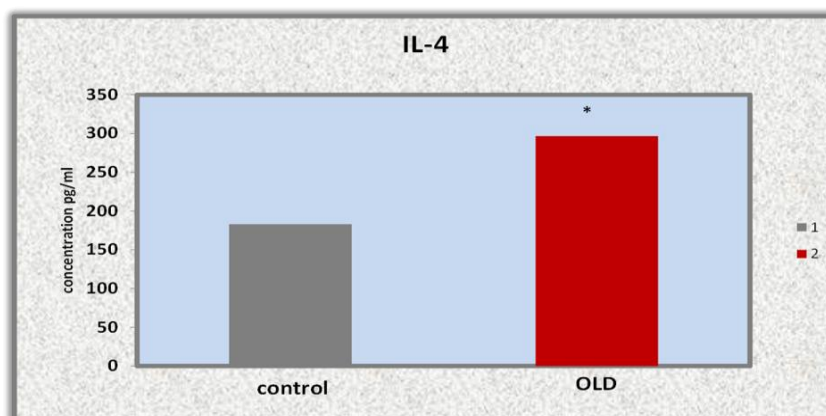
This study aims to investigate the relation between antioxidants levels and the development of obstructive lung diseases (OLD), in addition to investigating the level of IL4.

The results in figure (1) showed no significant change ( $P>0.05$ ) in GPX concentration in serum of patient with OLD group compared to the control group. The data show that the mean  $\pm$  SD of GPX in OLD and control group were  $749.206 \pm 57.63$  and  $740.479 \pm 84.427$  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  $\pm$  SD of IL-4 in OLD and control group were  $296.76 \pm 247.66$  and  $182.89 \pm 219.418$  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  $\pm$  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  $\pm$  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,<sup>(16)</sup> and in all study participants (OLD patients, control smokers, control nonsmokers).<sup>(17)</sup> A study by Biljak<sup>(18)</sup>, 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.<sup>(18)</sup>

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<sup>(19)</sup> which indicated that IL-4 marker of allergic airway inflammation.<sup>(19)</sup> Expression level of IL-4 was also significantly increased in asthmatic patients and highly correlated within individual subjects<sup>(20)</sup>.

Studies of IL-4 have revealed a wealth of information on the diverse roles of this

cytokine in homeostatic regulation and disease pathogenesis.<sup>(21,22)</sup> 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.<sup>(23,24)</sup>, 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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