

## **Determination of Oxidative Stress and Antioxidant System in Chronic Obstructive Pulmonary Disease (COPD)**

\*Abbas J. Kadhum \*Zainab N. AbduAllah

\*Bassam Faron Abed

Science College, AL-Qadisiya University. \*

### **Abstract :**

Airways are exposed to high levels of environmental oxidants, yet they also have enriched antioxidants. Airways disease such as chronic obstructive pulmonary disease (COPD) has evidence of increased oxidative stress and decreased antioxidants. The aim of this study was to examine oxidant/ antioxidant status in 45 patients with COPD (Age range: 30 to 50 years) and in (40) healthy subjects. The status of oxidants in serum as represented by malondialdehyde (MDA) levels increased significantly ( $p < 0.01$ ) in the conditions of chronic obstructive pulmonary disease (COPD), than their controls, also, non-enzymatic antioxidants (vitamin E, albumin and uric acid) which protects the lung from increased oxidative stress, were estimated in both of patients and controls groups. Vitamin E levels in patients with COPD were found to be significantly lower ( $p < 0.01$ ) as compared to control, On the other hand, low serum albumin ( $p < 0.001$ ) and uric acid levels were observed in the study group than controls. The changes in oxidant/ antioxidant status may be the effect of chronic obstructive pulmonary disease state.

### **الخلاصة :**

يتعرض مسلك الهواء (إلى الرئتين) إلى مستويات عالية من المؤكسدات البيئية، لكنه أيضا غني بمضادات الأكسدة. إن من أمراض المسلك الهوائي هو مرض الرئتين المعاق المزمن (COPD) حيث وجد فيه زيادة شدة الأكسدة ونقصان مضادات الأكسدة هدف هذه الدراسة هو اختبار مستوى الأكسدة / مضادات الأكسدة في 45 مريض (COPD) تتراوح أعمارهم (30- 50 سنة) و40 من الأصحاء. وضعية المؤكسدات في البلازما والتي تتمثل بمستويات المألون داي الديهايد (MDA) تزداد بشكل معنوي ( $p < 0.01$ ) في المرضى مقارنة بالأصحاء وكذلك تم قياس مستويات مضادات الأكسدة غير الإنزيمية (فيتامين E، الألبومين وحامض اليوريك) والتي تقوم بحماية الرئة من زيادة مستوى شدة الأكسدة. لقد وجد نقصانا معنويا في مستوى فيتامين E ( $p < 0.01$ ) في المرضى المصابين (COPD) مقارنة بمجموعة الأصحاء. من جهة أخرى لوحظ نقصانا في مستوى الألبومين ( $p < 0.001$ ) ومستوى حامض اليوريك في مجموعة المرضى مقارنة بالأصحاء. إن التغييرات في مستوى الأكسدة / مضادات الأكسدة من المحتمل أن يكون بتأثير الحالة المرضية المزمنة للرئتين.

### **Introduction :**

Chronic obstructive pulmonary disease (COPD) represents a major health problem and its prevalence and mortality rates are increasing worldwide (Pauwels et al. 2001) Chronic obstructive pulmonary disease (COPD) is a term referring to two lung diseases, chronic bronchitis and emphysema, that are characterized by obstruction to airflow that interferes with normal breathing. Both of these conditions frequently co-exist, hence physicians prefer the term COPD. It does not include other obstructive diseases such as asthma (Pauwels et al. 2002). It is the forth-leading cause of chronic morbidity and mortality. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lung to noxious particles and gases (Snez`ana, 2005). Smoking is the primary risk factor for COPD, One puff of smoke contains 10 14-16 free radicals (Kinnula and Crapo 2003). Approximately 80 to 90 percent of COPD deaths are caused by smoking. Female smokers are nearly 13 times as likely to die from COPD as women who have never smoked. Male smokers are nearly twelve times as likely to die from COPD as men who have never smoked (Mannino et al, 2000). Other risk factors of COPD include air pollution, history of childhood respiratory infections and heredity. Occupational exposure to certain industrial pollutants also increases the odds for COPD ( Hnizdo et al, 2002). Moreover, due to its anatomical and functional characteristics, the lung is an organ at high risk to oxidative damage, since it is

directly exposed to toxic substances deriving from air pollution, cigarette smoke and infective agents. Oxidative stress is also thought to play a role in many lung diseases, including lung cancer, asthma and chronic obstructive pulmonary disease (Takeyama et al., 2000). In the resting state, the balance between antioxidants and oxidants is sufficient to prevent the disruption of normal physiologic functions; however, either increases in oxidants or decreases in antioxidants can disrupt this balance. The state of imbalance is collectively referred to as oxidative stress and is associated with diverse airway pathologies (Halliwell & Gutteridge, 1999). An oxidant/antioxidant imbalance (oxidative stress) is thought to play an important role in the pathogenesis of chronic obstructive pulmonary disease (COPD)( Kluchova et al.,2007) Oxidative stress is thought to play an important role in the pathogenesis of COPD, not only through direct injurious effects, but also by involvement in the molecular mechanisms that control lung inflammation. The consequences of oxidative stress include oxidative inactivation of antiproteases, increased sequestration of neutrophils in the pulmonary microvasculature, gene expression of proinflammatory mediators, and airspace epithelial injury (Snez`ana, 2005). The major oxidants in airways are reactive oxygen and reactive nitrogen species (ROS/RNS) (Ottonello et al, 1995). Antioxidants are the primary defense against ROS/RNS. The antioxidant effect can be either enzymatic or nonenzymatic. Antioxidant enzymes include the families of superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione S-transferase, and thioredoxin. The nonenzymatic antioxidants include low molecular weight compounds such as glutathione, ascorbate, urate, ( $\alpha$ -tocopherol), bilirubin, and lipoic acid. Concentrations of these antioxidants vary depending on both the subcellular and anatomic location (Van der Vliet et al, 1999). Other high molecular weight molecules that might be considered antioxidants include proteins that have oxidizable thiol groups such as albumin or proteins that bind free metals such as transferrin. Albumin and transferrin are found in high concentration in serum but are in a much lower concentration in airway lining fluid. Thus, both the lung parenchyma and airways have several antioxidant systems (Reynolds HY& Newball, 1974). A number of antioxidant disturbances have been observed in patients with COPD. Lipid peroxidation products, one of the key indicators of oxidative stress (MacNee 2005), are elevated in sputum and exhaled breath condensate of patients with COPD of oxidative stress are increased even further during exacerbations of COPD, and in patients with very severe form of this disease (Kostikas *et al.* 2003). At the same time, the antioxidant mechanisms are attenuated in these patients, as indicated by anti oxidant vitamin in the lungs (Drost *et al.* 2005), The aim of the present study was, to study the serum oxidant and some nonenzymatic antioxidant status in patients with COPD and values were compared with normal healthy controls.

### **Materials and Methods :**

This study was conducted in 45 diagnosed cases of chronic obstructive pulmonary disease (COPD) ,and 40 healthy subjects non-smokers without any history of lung disease and had normal pulmonary function tests as control. The subjects were in the age group of 30 to 50, they were followed at AL-diwaniya hospital. The diagnosis of COPD in this study was established according to clinical examination. Subjects were selected after each patient had been assessed clinically. Pulmonary function tests were performed in (COPD) patients and in control subjects. Patients with any kidney, diabetes, liver diseases and pregnancy were excluded from the study. Ten milliter of blood was drawn by venipuncture and collected in a vial without EDTA (exact at the 8:00 a.m). Serum was separated and the samples stored at 4 °C till being processed. Samples were used for the estimations of serum MDA, vitamin E albumin and uric acid. Serum vitamin E levels were measured by high performance Liquid chromatography according to the method of Baker and Frank, 1988. Serum albumin concentration was estimated by (Doumas *et al.*, 1971) method. And the level serum of uric acid was measured by enzymatic colorimetric method (Henry, 1974 and Morin, 1973). Serum MDA was estimated in terms of thiobarbituric acid reactive species (TBARS) by the method of Fong *et al.*, 1973 the thiobarbituric acid reaction is used to measure serum MDA. In this test, the chromogen is formed by the reaction of one molecule of MDA with two molecules

of TBA. The method involves heating the sample (serum) with trichloroacetic acid and thiobarbituric acid under acidic conditions and reading the absorbance of the MDA-TBA adduct at 532 nm.

**Statistical analysis:**

was carried out using unpaired 't'- test student. SPSS program version 10 was used for statistical analysis. P value less than 0.05 ( $P < 0.05$ ) was considered as significant.

**Results and Discussion**

Results are summarized in (Table1). Numerous studies have shown that oxidative stress is increased in the lungs of patients with COPD compared to healthy subjects (MacNee 2005). Lipid peroxidation products are elevated in sputum, exhaled breath condensate (Tsukagoshi et al. 2000, Montuschi et al. 2000) and plasma of patients with stable COPD (Dekhuijzen, 2004). Moreover, exacerbations of COPD lead to even further elevations in various markers of oxidative stress (Kostikas et al. 2003). In addition, the oxidant/antioxidant balance is deteriorated further by the depletion of antioxidant mechanisms. Indeed, deficiencies in both enzymatic and nonenzymatic antioxidative systems were described in patients with COPD, (Rahman et al. 2000 & Drost et al. 2005). Vitamin E is a lipid soluble chain breaking antioxidant (Heunks. and Dekhuijzen, 2000). It converts  $\bullet O_2^-$ ,  $\bullet OH$  and lipid peroxy radicals to less reactive forms (Heffner and Repine, 1989). In this study table(1) shows significantly lower serum vitamin antioxidant (vitamin E) ( $0.93 \pm 0.1$  mg/dl) levels in patients group (COPD) as compared to control subjects. The results of this study confirm previous observations that there is low plasma antioxidant (vitamin E) in (COPD) patients (Zhou et al, 1997). Considerable evidence now links COPD with increased oxidative stress and, therefore, the status of antioxidant defense mechanisms assumes paramount importance (Kinnula and Crapo 2003). On the other hand serum albumin levels were lower ( $3.97 \pm 0.07$  g/dl) than normal subjects (table 1). This decrease in serum albumin is in agreement with many studies in the mentioned diseases which considered albumin as one of the antioxidants (Rowley and Halliwell, 1983). In normal healthy control, albumin is an important chain breaking extracellular antioxidant (Mee – Kyuing and II-Han, 1996) which has several biological functions, particular as a ligand binder (Sengupta *et al.*, 2001). Since albumin is the main source of the thiol group in plasma (estimated to be as high as  $500 \mu\text{mol/L}$ ). Thiol group, on the surface of albumin, bind oxidant. Albumin provides the bulk of "total plasma thiols". Although the thiol groups are oxidized during oxidative stress (Sengupta *et al.*, 2001). Thus, under these conditions, free radicals mediated oxidation and poor degradation of albumin may lead to accumulation of oxidatively modified albumin with lowered capacity to bind uraemic toxins and other protein bound substances (Jasmina *et al.*, 2001). Low level of albumin can cause oxidative stress via leading to increase oxidants like homocysteine (Sengupta *et al.*, 2001). Uric acid has a strong antioxidant activity and its concentration in the plasma is about ten fold than antioxidants like vitamin C and vitamin E (Ghiselli *et al.*, 2000). Our study (table 1) was showed relatively low serum levels of uric acid ( $4.92 \pm 0.36$  mg/dl) in subject with (COPD). Couillard *et al*, 2002 proved that no significant deficiency in plasma nonenzymatic antioxidants, as reflected by level of uric acid, was noted in the COPD patients compared with the healthy subjects. One of the mechanisms by which oxidants can cause lung injury, is lipid peroxidation. Malondialdehyde is the principal and most studied product of polyunsaturated fatty acid production (Del Rio et al, 2005). In the present study MDA was found to be having statistically significantly higher values ( $0.69 \pm 0.6 \mu\text{mol/l}$ ) in COPD patient group as compared to control group (table 1). Similarly, Lee 1997 reported increased lipid peroxidation products in patients with (COPD). Oxidative stress is a feature of most airways diseases, particularly when inflammation is prominent. Both an increase in ROS/RNS and depletion of antioxidants are thought to contribute the pathogenesis of oxidative stress (Russell and James, 2002). Considerable evidence links chronic obstructive pulmonary disease (COPD) with increased oxidative stress (Couillard *et al*, 2002). This study is agreement with study of Madhiparija et al., 2005 who suggested that high plasma MDA may be associated with lung function in patients with severe COPD. These observations indicate

that lipid peroxidation is markedly increased in patients with severe COPD. Thus, there is a recent study indicates that lipid peroxidation is more active in patients with more severe COPD suggesting that increases in toxic lipid peroxidation products might be related to the progression of the disease (Kluchova et al., 2007)

**Table1. Serum MDA and antioxidants levels in the control group and patients with COPD**

Groups	MDA (µmol/l)	Vitamin E (mg/dl)	Albumin (g/dl)	Uric acid (mg/dl)
<b>Controls (n=40)</b>	0.48±0.055	1.27±0.22	4.83±.98	5.32±0.68
<b>COPD (n=45)</b>	0.69±0.06	0.93±0.1	3.97±0.07	4.92±0.36

Values expressed as (Mean ± SD) with  $p \leq 0.05$ .

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