



STUDY OF SERUM OXIDANT-ANTIOXIDANTS STATUS IN ADULT BRONCHIAL ASTHMATICS

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Abstract

Asthma is a chronic inflammatory airway disorder associated with recruitment of inflammatory cells. Asthmatic patients produce reactive oxygen species damaging the antioxidant defense system and forming a state of oxidative stress in asthmatics. The present study included 50 patients [25 males and 25 females] and 50 supposed healthy subjects (control) [25 males and 25 females]. The results show a presence of a significant increase ($p \leq 0.05$) in malondialdehyde (MDA) and ceruloplasmin (Cp) levels in both sexes of bronchial asthmatic (BA) patients in contrast with the control group. On the other hand, transferrin (Tf) showed a significant decrease ($p \leq 0.05$) in male BA patients compared with the control group, whereas, serum Tf levels demonstrated a non-significant decrease among the female BA patients in comparison with the control group. Correlation coefficient (r) test is used to describe the association between lipid peroxidation products and different antioxidants. This study revealed a significant negative correlation between MDA and the levels of Cp in both male and female BA patients ($r = -0.74$ and $r = -0.37$ respectively). Similarly, there was a significant negative correlation between MDA and Tf in male patients ($r = -0.89$), whereas the comparison between MDA with Tf revealed non-significant correlation in female BA patients. Asthma leads to a considerable imbalance between antioxidants and oxidants. Both sexes of BA patients undergo an extraordinary degree of reactive oxygen species (ROS) development triggering considerable oxidative stress indicated by high levels of lipid peroxidation marker MDA and antioxidant Cp and low level of the antioxidant Tf in both male and female BA patients.

Keywords: *Bronchial Asthma, Malondialdehyde, Ceruloplasmin and Transferrin*

Introduction

Asthma may be observed as a diffuse obstructive lung disease with hyper-reactivity of the airways to a range of stimuli and a high degree of reversibility of the obstructive process, which may follow either spontaneously or as a result of treatment (1). When people talk about bronchial asthma, they are definitely talking around asthma, a common chronic inflammatory disease of the airways that causes the airway of the lungs to swell and narrow leading to coughing, shortness of breath, wheezing, chest tightness and excess mucus production (2). Asthma is a complex condition involving biochemical, autonomic, immunologic, infectious, endocrine, and psychological factors in variable degrees in different persons; it can be prevented by avoiding causes, such as irritants and allergens (3). Most persons can develop a severe exacerbation of asthma from various triggering agents. Bronchial asthma triggers may include smoking and secondhand smoke; infections such as flu, colds, or pneumonia; allergens such as food, mold, pollen, mites, dust and pet dander; exercise; air pollution and toxins; weather, mainly great changes in temperature; drugs (such as aspirin and beta-blockers); food additives; emotional tension and anxiety; singing, crying, or laughing; perfumes and fragrances and acid reflux. Both virus and bacterial infections of the upper respiratory tract infection can worsen asthma (4). Several mechanisms operate in cellular damage and death, lipid peroxidation caused by free radicals being one of the most important mechanisms. A free radical is an atom or molecule that has one or more unpaired electrons, they are two types; a reactive oxygen species (ROS), such as superoxide anion ($O_2^{\bullet-}$), peroxide anion ($O_2^{\bullet-2}$), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^{\bullet}), and hydroperoxyl radical (HO_2^{\bullet}); and a reactive nitrogen species (RNS), such as nitric oxide (NO^{\bullet}), nitrogen dioxide (NO_2^{\bullet}), peroxyxynitrite ($OONO^{\bullet}$) (5). Because ROS are so reactive, they can inflict considerable damage on living cells if formed in significant quantities. These damage results primarily from enzyme inactivation, polysaccharide depolymerization, DNA breakage and membrane destruction (6, 7). Oxidative stress doesn't only cause direct harmful effects in the lungs but also activates molecular mechanisms that recruit lung inflammation (8). Thus, an imbalance between oxidants and antioxidants is considered to play an essential role in the pathogenesis of asthma. A lot of oxygenated compounds, particularly aldehyde such as MDA, are produced during the attack of free radicals to membrane lipoprotein and polyunsaturated fatty acid, products of lipid peroxidation formed in the primary site. These products reach the other organs and tissues via the blood stream provoke other lipid peroxidation and cause cellular and tissue damage (9). The Increase of lipid peroxidation could possibly play a role in the complication of cardiovascular disease, chronic pulmonary disease, cataract and cancer (10,11). Oxidative stress occurs when this balance is disrupted by extreme production of reactive oxygen species and / or by insufficient anti oxidative defenses, including superoxide dismutase, catalase, vitamins C and E, β -carotene, uric acid, glutathione and trace elements such as zinc, selenium, magnesium, copper, iron, which are cofactor for many biochemical reactions (12, 13,14). Ceruloplasmin (E.C.1.16.3.1) is a multicopper enzyme carrying around 95% of circulating copper (15). Cp is an important extracellular antioxidant through ferroxidase activity, it also scavenges superoxide anion radical ($O_2^{\bullet-}$). Ceruloplasmin with its ferroxidase activity can oxidize ferrous ions to the less toxic ferric form without releasing reactive oxygen species, causing protect the body from the potentially damaging effects of various oxidizing agents (16, 17, 18). Increased plasma ceruloplasmin levels are associated with the generations of oxidation products, i.e., $O_2^{\bullet-}$ and H_2O_2 (19).

The aim of this study is to investigate the oxidative stress by evaluating MDA in patients with BA disease, and to determinate serum antioxidant status (by measurement Cp and Tf) compared with the control group, then to shed a light on the possible correlation between MDA and each of Cp and Tf.

Materials and Methods

Design of Study

This study was conducted at AL-Hussein Teaching Hospital in AL-Muthanna governorate (Iraq) at the period 10/3/2014 to 15/7/2014. The study includes 100 subjects, fifty patients with bronchial asthma [25 males and 25 females] with an age range [15- 65] years for male and female. The control group included 50 subjects [25 males and 25 females] with an age range [15-68] years, healthy, non-smokers without any history of lung disease and had normal pulmonary function tests.

Blood Sample Collection

From the patients with bronchial asthma and the control groups, 5mL blood sample was taken. Blood samples were collected from each subject by vein puncture, centrifuged at 3000 rpm for 10 min after permitting the blood to clot at room temperature. The serum was divided into two equal parts in sample tubes, serum MDA were tested within 24 hours, whereas the others were stored at -20°C till estimating Cp and Tf.

Lipid peroxidation Marker in Bronchial Asthma (Serum MDA)

Lipid peroxidation as serum MDA level was estimated in terms of thiobarbituric acid reactive species (TBA) (20). In this investigation, the chromogen is formed by the reaction of one molecule of MDA with two molecules of TBA. The process involves warming the sample (serum) with TBA and trichloroacetic acid, then reading the absorbance of (MDA-TBA) adduct at 532 nm.

Serum Antioxidants

Serum Cp concentration was measured by the method of Menden at 525 nm using para-phenylenediamine as a reagent (21). Transferrin (Tf) is the most important extracellular iron transport protein in humans (22). Transferrin concentration was measured by colorimetric method includes saturation of Tf with an excess predetermined iron concentration, removal of unbound iron by magnesium carbonate as a precipitating agent, then measurement of the iron-binding Tf (23). After centrifugation at 3000 rpm for 10 min, the iron in the supernatant is determined at 600 nm (24). The total iron binding capacity (TIBC) of human serum is considered to be a useful estimation of its transferrin concentration. The serum Tf concentration was calculated by the following equation (25).

$$(\text{Serum Tf (gm/L)}) = (0.094 \times \text{TIBC (}\mu\text{g/dL)}) - 32.8$$

Statistical Analysis

Statistical analysis was performed using Statistical Package Social Science SPSS version 15.0. Quantitative data were summarized as mean \pm standard deviations values obtained for AP patients and controls. The one way analysis of variance (ANOVA) is a technique used to determine whether there are any significant differences between the means of different studied group. P-values ($P \leq 0.05$) are taken as the level of significance. Further, Pearson's correlation (r) was used to correlate between the different parameters in each patient group.

Results and Discussion

Asthma is a chronic inflammatory disease of the respiratory tract of unknown etiology. According to the world health organization, asthma is regarded a serious public health problem with over 100 million sufferers worldwide, death from this condition has reached over 180,000 annually (26,27). Oxidants-antioxidants balance is essential for the normal lung function. Both, an increased oxidant and/or decreased antioxidant may reverse the physiologic oxidant-antioxidant stability in favor of oxidants, leading to lung injury. A number of diseases involving the lung, such as emphysema, bronchiectasis and bronchial asthma have balances (28). The present study revealed a highly significant elevation ($P \leq 0.05$) of MDA among all BA patients studied compared to healthy control in both males and female patients (Table 1), indicating thereby the relation of serum MDA level with that of the underlying inflammatory process in bronchial asthma. These imply that patients during asthmatic attack are wide-open to a great degree of lipid peroxidation. This result is consistent with the observations of others (11). MDA is a pointer of lipid peroxidation, it has a strong correspondence with atopic asthma, proposing that oxidative stress occurs simultaneously on lipid peroxidation. Oxidative stress can have different special effects on the function of airway, including contraction of airway smooth muscle, induction of airway hyper responsiveness, hyper secretion of mucus, vascular exudation and epithelial shedding. Moreover, ROS can induce the production of cytokine and chemokine, through the initiation of oxidative stress-sensitive transcription on nuclear impact in bronchial epithelial cells includes the consumption of oxygen and consecutive release of ROS into adjacent cells (29). During the respiratory burst, the inflammatory cells have released high concentration of $O_2^{\bullet-}$, OH^{\bullet} , $HOCl$ and H_2O_2 that may leak into adjacent cells resulting in increased amounts of free radicals in airway tissues. Moreover, the inflammatory cell of asthmatics has an increased capacity to create free radicals, which further contributes to high concentrations of ROS. One marker of airway inflammation in the asthmatics is the excess generation of reactive nitrogen species (RNS) (30). Cytokines may excite increased production of nitric oxide radical (NO^{\bullet}) which reacts with $O_2^{\bullet-}$ to form peroxynitrite ($ONOO^-$), a cytotoxic species that has many destructive effects, including lipid peroxidation. Therefore, the additional amounts of ROS and RNS that are produced by asthmatics may overcome the host antioxidant defenses and cause oxidative stress (31, 32).

Table 1: Serum level of malondialdehyde in BA patients and healthy control classified according to sex.

Groups	n	MDA(nmol/mL) Mean \pm SE	
		Female	Male
Control	25	14.63 \pm 0.35 ^b	13.72 \pm 0.49 ^b
Patients	25	338.00 \pm 10.38 ^a	405.50 \pm 9.14 ^a

The above values represent mean \pm SD values with non-identical superscript (a, b) were differ significantly ($p \leq 0.05$).

Ceruloplasmin

The oxidant/antioxidant imbalance has been proposed to play important role in the pathogenesis of asthma. However in the current study, serum ceruloplasmin level was increases significantly ($p \leq 0.05$) in both males and females BA patients as compared with normal subjects (Table 2). The present study is in agreement with another study (33). Increased ceruloplasmin levels were demonstrated in asthma patients (34). Levels of certain proteins in plasma rise through acute inflammatory state or according to certain types of tissue damage. These proteins are named acute phase proteins or reactants. They are synthesized by the liver in response to the mentioned cases. Following stimulus through infection or injury, the macrophages liberate monokine and interleukin-1, which induce the liver to secrete a lot of acute phase proteins. These reactants have been shown to serve different effective roles during tissue rehabilitation of injury or inflammation in various mechanisms of host immune defense. Cp is recognized to keep considerable ferroxidase activity and capable of suppression superoxide radicals. It is responsible for restricting the damage caused by these radicals (35).

Table 2: Serum level of ceruloplasmin in BA patients and healthy control classified according to sex.

Groups	n	Ceruloplasmin (g/L) Mean \pm SE	
		Female	Male
Control	25	0.275 \pm 0.13 ^b	0.284 \pm 0.13 ^b
Patients	25	1.899 \pm 1.02 ^a	2.437 \pm 1.64 ^a

-Legend as in table (1)

Transferrin

The presence of inflammatory cells such as eosinophils, mast cells, macrophages and lymphocytes has been observed in different specimens of asthmatic patients. These inflammatory cells secrete several kinds of cytokines (36). Therefore, asthma and allergic inflammation include wide cytokine network. This cytokine and their complex relationship have an important role in inflammation and immune. The acute - phase reaction is a general response to inflammation and probably triggered by interleukins, released from the site of injury or inflammation. Interleukin-2, interleukin-6 and tumour necrosis factor- α are an important mediator in the synthesis of amyloid precursor protein by liver (37). In the present study, transferrin level was significantly lower ($p \leq 0.05$) in male BA patients compared with the control group. A similar result was observed by Mukadder (33). Similarity, Bakkeheim (38) found that there was a significant decrease ($p \leq 0.05$) in transferrin levels in asthmatic patients. In consistence with other observations showed no difference was observed in transferrin levels between the groups (34). Our study revealed non-significant decrease of Tf serum level among the BA female patients in comparison with the control group (Table 3).

Table 3: Serum level of transferrin in BA patients and healthy control classified according to Sex.

Groups	n	Transferrin (g/L) Mean \pm SE	
		Female	Male
Control	25	3.39 \pm 0.12 ^a	3.52 \pm 0.13 ^a
Patients	25	2.61 \pm 0.25 ^a	1.74 \pm 0.39 ^b

-Legend as in table (1)

Correlation coefficient (r) between oxidant and antioxidant was studied for both male and female patient groups. The results of this study illustrate the presence of a significant negative correlation between Cp and MDA ($P < 0.01$) in a male patient ($r = -0.74$) (Figure 1), also there was a significant negative correlation between Cp and MDA ($P < 0.05$) in female patients ($r = -0.41$) (Figure 2). However, there was a significant negative correlation between transferrin and MDA ($P < 0.01$) in a male patient ($r = -0.89$) (Figure 3). Similarity comparison between transferrin with MDA revealed a significant negative correlation ($P < 0.01$) in female (BA) patients ($r = -0.86$) (Figure 4).

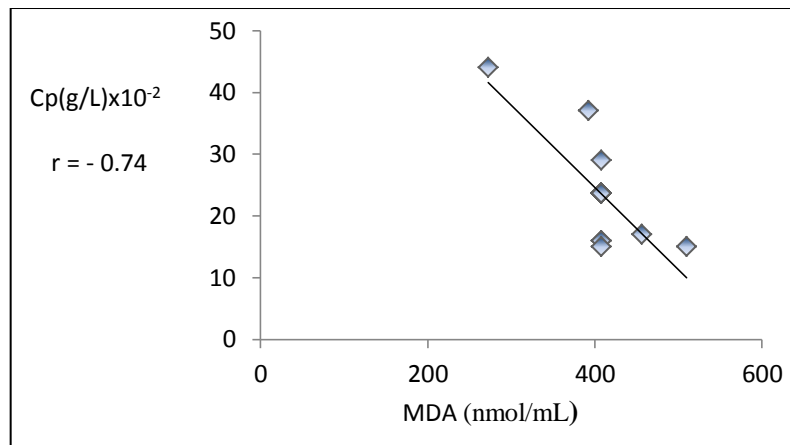


Figure 1: Correlation between MDA and Cp in male BA patients

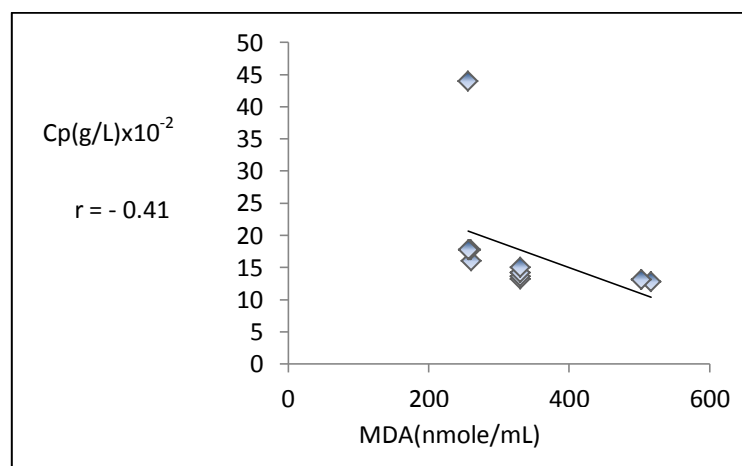


Figure 2: Correlation between MDA and Cp in female BA patients.

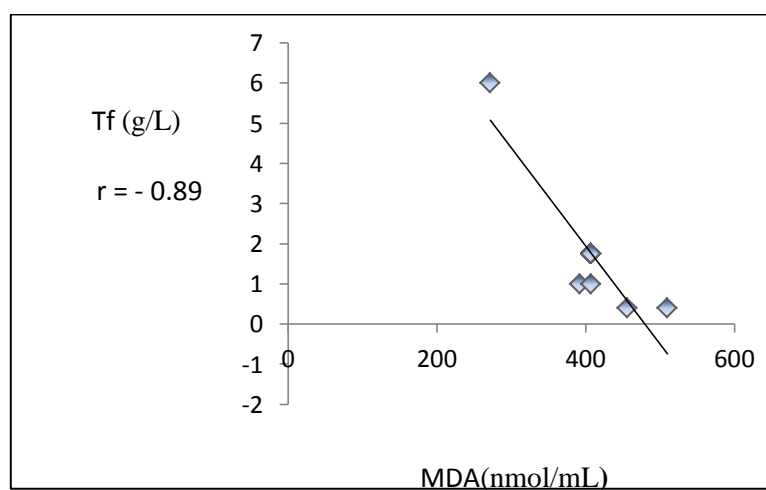


Figure 3: Correlation between MDA and Tf in male BA patients.

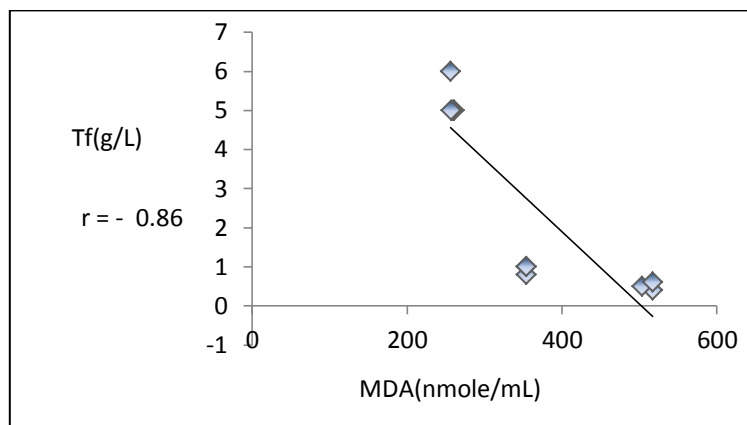


Figure 4: Correlation between MDA and Tf in female BA patients.

Conclusion

Bronchial asthmatic patients have increased lipid peroxidation level as detected by the increase in serum MDA level, which was an important factor in increasing the production of free radicals that developed bronchoconstriction. As a result of continuous production of reactive oxygen species the antioxidant Tf level was significantly decreased, while antioxidant Cp increased.

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