Evaluation of Superoxide Dismutase and Malondialdehyde in Asthmatic Patients in Al-Hilla Province

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Abstract

The present study was conducted to explore the oxidative stress levels in asthma. To achieve this aim, 87 asthmatic patients, and 46 healthy individuals were enrolled in this study. The samples were obtained from Babylon Asthma and Allergy center in Hilla city. The laboratory work was carried out in the research-lab of the departments of biochemistry, college of medicine, Kufa University, Iraq. Superoxide dismutase activity and malondialdehyde concentrations were determined spectrophotometrically. The used statistical analysis (student`s t-test) showed a highly significant ($p<0.001$) decrease in superoxide dismutase and a highlysignificant ($p<0.001$) increase in malondialdehyde concentrations in asthmatic patients when compared with healthy individuals. The linear regression analysis demonstrated a significant negative correlation for superoxide dismutase ($r = -0.63$, $p<0.01$), and a significant ($r = 0.53$, $p<0.01$) positive correlation for malondialdehyde concentration with age of asthmatic patients. The statistical analysis indicated a significant negative correlation for superoxide dismutase ($r = -0.63$, $p<0.01$) and a significant ($r = 0.44$, $p<0.01$) positive correlation for malondialdehyde concentration with the duration of asthma. Patients from rural areas showed a significant ($p<0.01$) decrease of superoxide dismutase and a significant ($p<0.01$) increase of malondialdehyde concentrations when compared to those of urban residency. The results indicated that asthma is highly associated with oxidative stress. It may evoke the addition of antioxidant to the treatment of asthma,And those who live in rural area should ingest a high amount of anti-oxidant to ensure the balance between the oxidant species and the well-done anti-oxidant in the cell.

Key words: superoxide dismutase, malondialdehyde, Iraq population.

تقدير انزيم السوبراوكسيد دسميوتيز وتركيز المالونداياالديهايد لدى مرضى الربو في مدينة الحلة

الخلاصة

أجريتْ الدراسة الحالية لاستكشاف مستوى إلاجهاد التاكسدي لدى مرضى الربو ولتحقيق هذا الهدفِ،تم سحب عينات الدم من 87 مريضا مصابون بالرَّبوِ و46 شخصا من الأصحاء. تم الحُصِول على العينات مِنْ مركزِ الربو و الحساسيةِ في بابل. اجري العمل ألمختبري في مختبرِ قسمِ الكيمياء الحياتيةِ- كليَّة الطبِّ- جامعة الكوفة- العراق.حيث تم تقدير الفعالية لإنزيم (superoxide dismutase) SOD والتركيز للمركب MDA (malondialdehyde) طيفيا. اجري التحليل الإحصائي باستعمال T-test(اختبار تي) الذي اظهر نقصانا معنويا ($p <0.001$) في مستوى لـ دى DOS مرضى الربو مقارنة بالأصحاء وتبين ان هنالك زيادة معنوية عالية($p <0.001$) في مستويات MDA لدى المرضى المصابون بالرَّبوِ عند مقَارنتهم مَع الأصحاء من نفس الفئة العمرية. اظهر تحليل الانحدار الخطي إرتباطا سلبيا معنويا ($r =-0.63$, $p <0.01$) بين العمر بالسنوات وقيم SOD وارتباطا ايجابيا معنويا ($r =0.53$, $p <0.01$) بين اعمار المرضى وتركيز MDA لديهم.كما أجريت تحاليل الانحدار الخطي الإحصائية بين متغيرات الإجهاد التاكسديMDAو SOD و مدة المرض بالسنوات حيث كان الارتبط إيجابي وملحوظ ($r =0.44$, $p <0.01$) لقِيَمِ MDA المصلية مع مدة المرض وسلبيا معنويا ($r = -0.63$, $p <0.0$) لقيم فـ عال يةSOD عند ربطها بمدة المرض.كما وتم تقصي مستويات الإجهاد التاكسدي لدى القرويين من المرضى الساكنينْ في المناطق الريفية النائية حيث اظهرت نتائجهم وجود نقصان معنوي ($p <0.01$) في مستوى فعالية الإنزيم SOD وزيادة معنوية ($p <0.01$) في تركيز MDA عند مقَارنتهاَ مع تلك المستقاة مِنْ ذوي الإقامةِ الحضريةِ. لقد دلت النَتائِجُ على أنّ الربو يُرتَبطُ بإلاجهادِ التاكسدي إلى حدٍ كبير.مما قد يستدعي إضافة مضادات الأكسدة الى

العلاج.وان على المرضى المقيمين في الأرياف الاهتمام بتناول كميات وافية من مضادات الاكسدة لضمان المحافظة على التوازن المنشود بين الاصناف المؤكسدة الضارة ومضادات الاكسدة محمودة الاثر.

Introduction

Oxidative stress occurs when the reactive oxygen species (ROS) overwhelm the antioxidant defences of the host. Oxidative stress plays an important role in the pathophysiology of asthma and may be a final common pathway leading to tissue damage.[1] Exposure to a variety of different substances, such as allergens, gaseous, pollutants, chemicals drugs, bacteria and viruses leads to the recruitment and activation of inflammatory cells in asthmatic airways including mast cells, eosinophils, neutrophils, lymphocytes and macrophages.[2] Activated inflammatory cells respond with a "respiratory burst" which involves the uptake of oxygen and subsequent release of reactive oxygen species into surrounding cells. During the respiratory burst, NADPH dependent superoxide generating system is activated and releases superoxide (O_2^-) into the cell.A dismutation reaction catalyzed by SOD then results in the production of hydrogen peroxide (H_2O_2) which in the presence of halide ions (i.e. Cl^- , Br^-) will react to form a hypohalous acids (e.g. HOCl, HOBr).[3]In eosinophil, this reaction is catalyzed by eosinophil peroxidase (EPO) . In neutrophils, this reaction is catalyzed by myeloperoxidase (MPO)

$$H_2O_2 + Cl^- + H^+ \xrightarrow{MPO} HOCL + H_2O$$

HOCl and HOBr may then react with O_2^- or Fe^{+2} to produce strong oxidant probably (OH^-)

$$HOCl + O_2 \text{ - } \longrightarrow {}^{\cdot}OH + Cl^- + O_2$$

$$HOCl + Fe^{+2} \longrightarrow {}^{\cdot}OH + Cl^- + Fe^{+3}$$

Thus during respiratory burst , the inflammatory cells have released high concentration of O_2^- , OH^- , HOCl , HOBr and H_2O_2 that may leak into surrounding cells resulting in increased quantities of free radicals in the airway tissues[4]. Furthermore, the inflammatory cells of asthmatics have an increased capability to generate free radicals compared to controls which further contributes to high concentration of ROS [5]. In addition to the recruited inflammatory cells, the constitutive airway cells such as epithelial cells are also potential sources of ROS.[6]

In addition to endogenous sources, some environmental factors linked to asthma such as air pollutants (e.g.ozone diesel exhaust particles) may cause an extreme increase in ROS generation in the airways.[7] Thus, the excess quantities of ROS that are produced by asthmatics may overcome the antioxidant defenses and cause oxidative stress. Oxidative stress can have many detrimental effects on airway function, including airway smooth muscle contraction,[8] induction of airway hyperres-ponsiveness [9]and mucus hypersecretion. [10]

Many studies suggested that ROS mediated reactions may alter or induce some inflammatory and immunological cellular responses for example through the generation of second messengers. If ROS are important in asthma, enhancement of antioxidant defenses would be expected to have beneficial effects in the disease. A diet rich in antioxidant such as selenium, vit E, vit C and β-carotene may be of help in alleviate asthmatic attacks in individuals exhibiting asthmatic symptoms.[11]

Materials and Methods

The cross sectional study was conducted on the following groups in

the period between September /2010 and March /2011. The samples were obtained from Babylon Asthma and Allergy center in Hilla city. Eighty-seven asthmatic patients (46 male and 41 female) of age 46±17(range 8-61) years were enrolled. Individuals who suffer from diseases like diabetes mellitus, hypertension, cardiovascular disease and liver diseases or those who using oral contraceptives, which interfere with the results of this study, were all excluded.

A questionnaire paper was designed to record the information of asthmatic patients. It contains many questions about the duration of the disease and patient age etc. Thirty three apparently healthy volunteers were included in this study. They were age matched with patients group. The laboratory work was carried out in the laboratory of research in the departments of biochemistry, college of medicine, Kufa University.

Disposable syringes and needles were used for blood collection. Blood samples were obtained from asthmatic patients and control group by vein puncture. Samples were allowed to clot at room temperature, and then centrifuged at 3000 xg for 10 minutes. Sera were transferred carefully and stored at -17 ^{o}C until analysis time in a suitable serum tubes. Superoxide dismutase activity was determined by superoxide dismutase assay kit purchased from Fluka company, Switzerland, while the concentration of malondialdehyde was determined by modified procedure described by Guidet B. and Shah S.V.in 2002 [12]Superoxide dismutase and malondialdehyde concentration results were analyzed using student's t-test.

Results

The results reveal that there is a significant ($p<0.001$) decrement in SOD activity in sera of asthmatic patients in comparison with control as shown in table 1.

Table 1 Superoxide dismutase (SOD), and malondialdehyde (MDA) concentrations in asthmatic patients and control group.

	subject	no.	mean±SD	range	p-value
SOD	control	33	86.3±7.0	74-95	<0.001
U/L	patients	87	67.3±17.8	44-89	
MDA	control	33	3.9±2.8	0.6-6.6	<0.001
µM	patients	87	7.6±4.3	2.2-13.5	

While the MDA concentrations in patients show a highly significant ($p<0.001$) enhancement when compared with the control group.

In figure 1 a linear regression plot was made between serum SOD activity and the age of the patients. The r value was -0.63 and p-value was <0.001 as shown below.

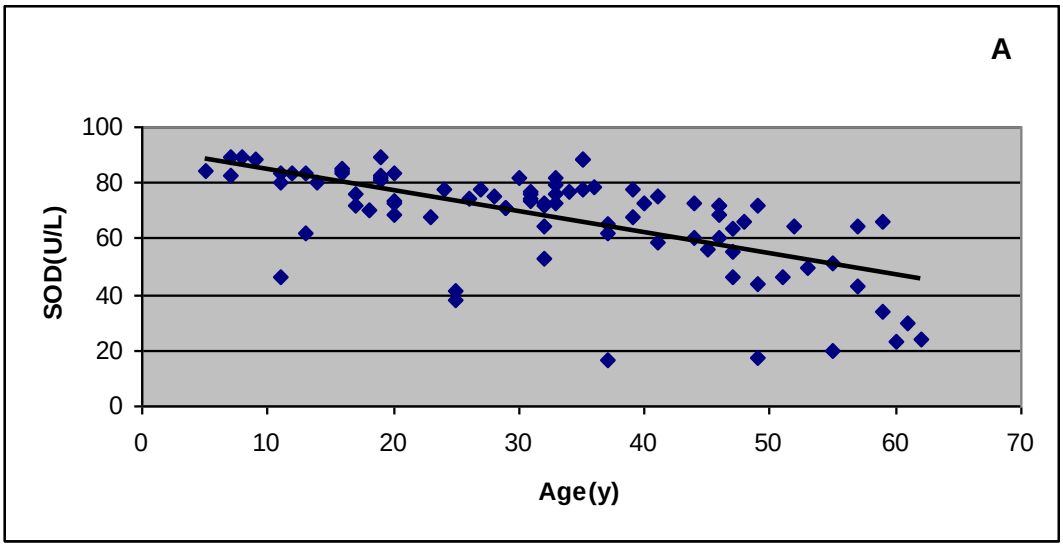

Figure 1 Correlation between serum SOD activity and ages of asthmatic patients.

In figure 2 below a linear regression plot was made between serum MDA concentrations and the ages of those patients the r value was 0.53 and the p-value was <0.001

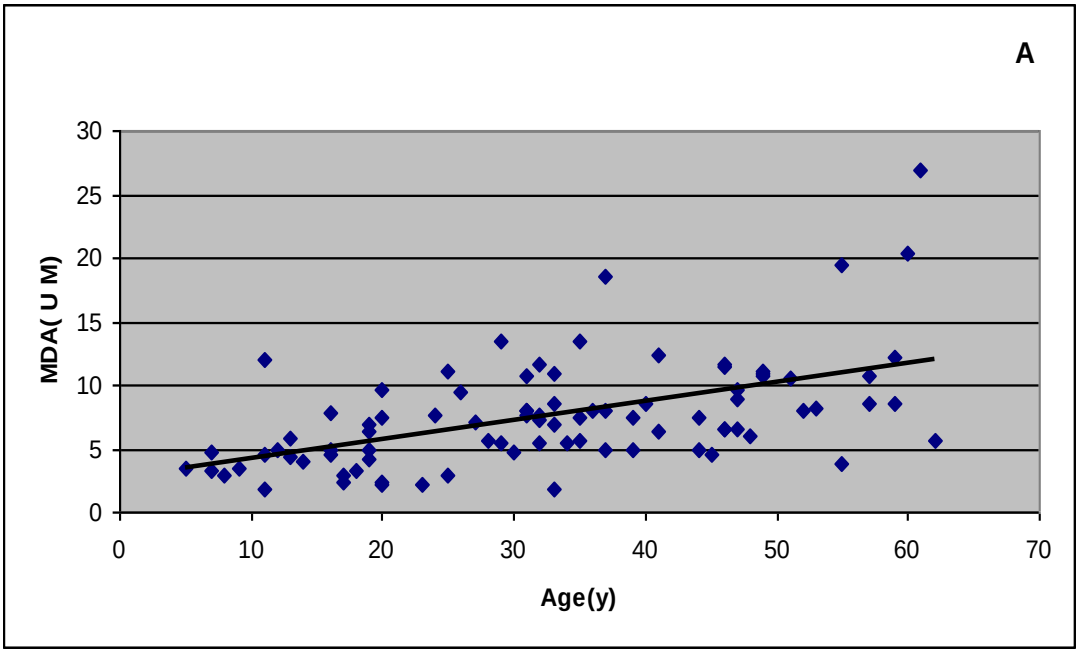

Figure 2 Correlation between serum MDA concentrations and ages of asthmatic patients.

The residency in rural and urban area was of special importance. Table2 shows the concentrations of oxidative stress parameters of patients inhabiting those areas.

Table 2 The effect of residency on superoxide dismutase activity, and malondialdehyde concentration in asthmatic patients

	residency	no.	mean±SD	range	p-value
SOD (U/L)	rural	45	62.8±19.5	44.1-87.2	0.01
	urban	42	73.7±11.9	53.5-89.4	0.01
MDA (µM)	rural	45	8.34±4.57	2.2-13.46	0.01
	urban	42	6.20±2.19	3.5-11.50	0.01

In figure 3 a linear regression plot was made between serum SOD activity and the duration of the disease the r value was -0.63 and p-value was <0.001 as shown below

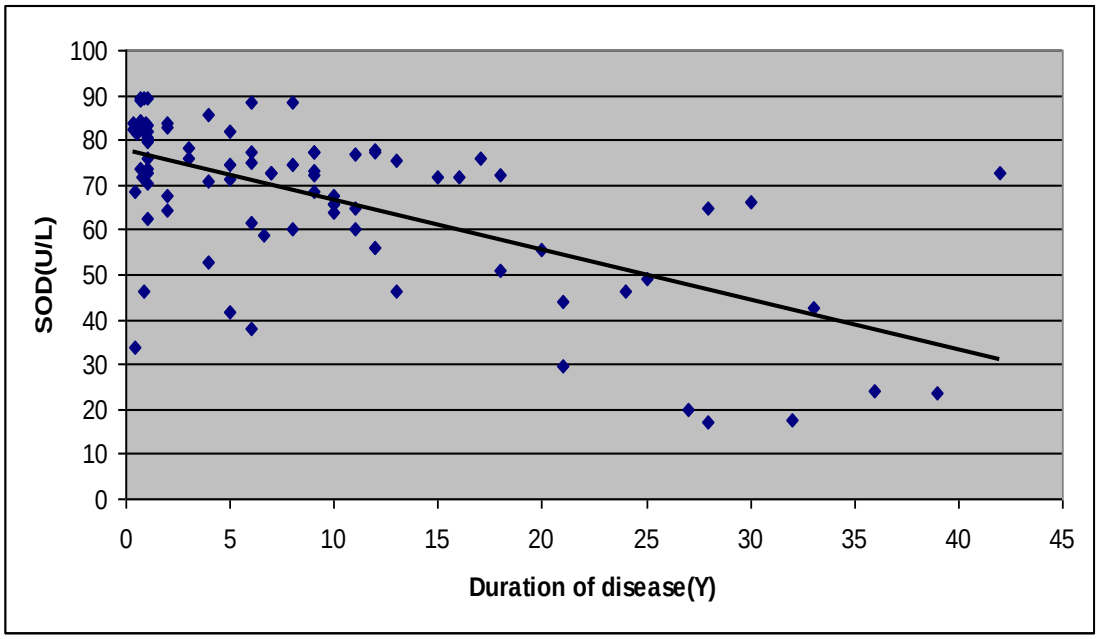

Figure 3 Correlation between serum SOD activitys and disease duration time of asthmatic patients.

Also in figure 4 a linear regression plot was made between serum MDA concentrations and the duration of the disease the r value was -0.44and p-value was <0.001 as shown below.

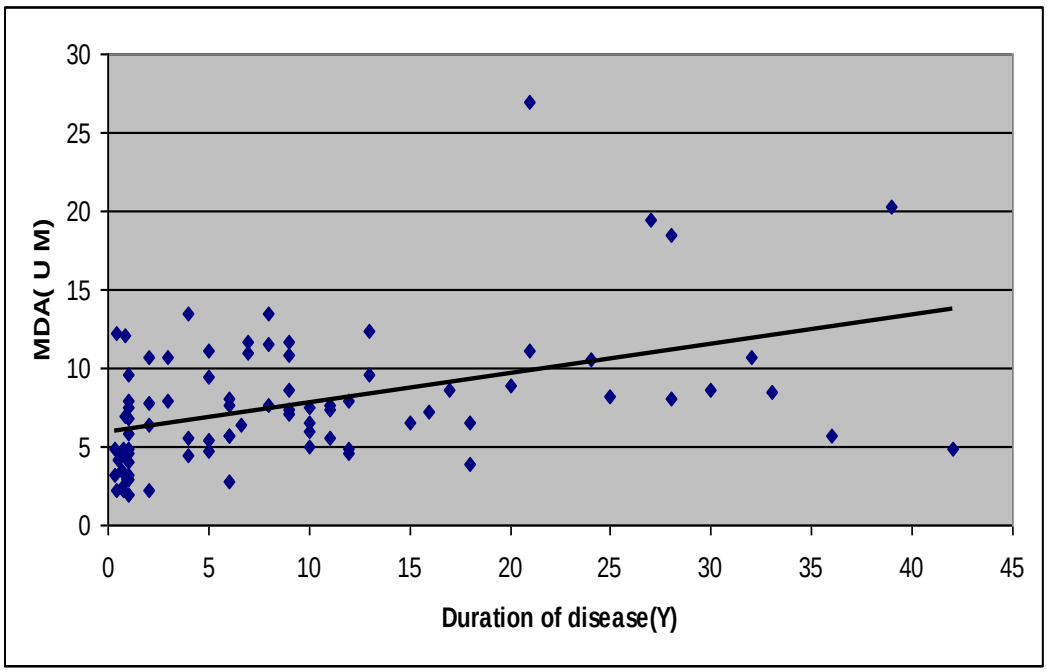

Figure 4 Correlation between serum MDA concentrations and disease duration time of asthmatic patients.

Discussion

The significant (P< 0.001) decrement in superoxide dismutase values in sera of asthmatic patients and the significant (p<0.001) enhancement in serum MDA concentrations in those patient as shown in table 1 is consonant with the mechanism of the disease. Asthma is a chronic inflammatory disorder and since there is a complex interaction of cells and mediators that result in an increased production and release of ROS.[13] It is hypothesized that the excess quantities of ROS that are produced by chronic inflammatory diseases may overcome the host antioxidant defenses and cause oxidative stress.[14]

In the present study, SOD activities were found to be decreased in asthmatic patients with respect to control group. These results are in consistence with the hypothesis of the deficiency for antioxidant in patients with asthma.[15]

Antioxidant enzymes like SOD are crucial for protection of the airway tracts against the deleterious effects of the oxidants. SOD activity is reduced in the oxidant – rich environments of the asthmatic airway and during asthma exacerbation, further loss of SOD activity may occur with enhanced production of oxygen radicals by inflammatory cells.[5,16] Comhair *et al*(2005) put forward that asthmatic individuals having greater amount of oxidative stress may have localized decrease in SOD activity. This may be reflected systemically in partial loss of circulating SOD activity.[14]

On the other hand, the lipid per oxidation product (MDA) concentration is increased significantly in sera of asthmatic patients when compared with those of the control group. The rise in MDA concentration is attributable to the increase generation of ROS owing to the excessive oxidative damage generated in these patients. The oxygen species in turn have the ability to oxidize many important biomolecules including membrane lipids. Similar reports show that MDA concentration elevated in patients with asthma.[17,18]

The linear regression analysis indicated a significant negative correlation between SOD values (r = -0.63, P <0.01) and ages of asthmatic patients (fig1),but it was positive with MDA (fig 2). The oxidative stress hypothesis of aging (or the free radicals hypothesis as it is first proposed) is currently one of the most popular explanations of how aging occur at the biochemical levels.[19] The basic tenet of oxidative stress hypothesis is that the age related loss of physiological functions are caused by progressive and irreversible accumulation of oxidative damage.[20] Individuals are exposed to oxidants, both exogenous as well as endogenous, from the birth and there is an increase in oxidant generation among older groups, affected them with a number of age – related degenerative diseases.[21,22,23]Some studies explain these results by put forward the suggestion that an increased production of ROS and / or a decreased efficiency of antioxidant defense system are associated with aging process.[24]

Aging seemed to be associated with increase in systemic oxidative stress where by-product of oxidative modification such as lipid peroxidation (MDA) accumulates within tissues. Elevated lipid peroxidation with aging in human are associated with decreased levels of endogenous antioxidants.[25]
The effect of populating area on SOD and MDA concentrations in asthmatic patients were shown in table 2. It reveals that there is a significant (P < 0.01) decrease in serum SOD and a significant (P < 0.01) elevation of serum MDA concentration in rural patients when compared with urban. Several explanations may be account for the enhancement of ROS in rural patients. The first is that the majority of those patients (more than half) were Iraqi farmers. They were subjected to exposure for so many organophospharus compounds and other type of pesticides especially due to the wrong way of applying these agents to the soil by making solutions in containers and throwing the solutions by their bare hands on the plants. Such compounds may be harmful to the cells and act as an oxidative stress stimulators.[26]
The low socioeconomic standard of those patients and their environment conditions make their diet generally rich with fats but with minimal fruits. This lifestyle raised the probability of ROS production.[27] They are also prone to a huge number of pollutants, where their lifestyle characterized by poor housing conditions, dampness (which encourages the growth of moulds and house – dust mites), use of polluting fuels for home heating and cooking without proper ventilation.
The linear regression analysis demonstrated a significant negative correlation of SOD values (r= -0.63, P <0.01), with the duration of disease of asthmatic patients .This analysis indicated a significant(r = 0.44, P < 0.01) positive correlation for MDA values with the duration of disease of asthmatic patients(Fig 2)(table 4). The results of the current study pointed out that the oxidative stress increase as the duration of disease increase. The duration of the asthma has been considered to exert a major influence on lung function. It is involved in the development of airway remodeling in asthma as a result of prolonged inflammation.[28]The factor that may play an essential role in consecrating the oxidative stress in these loci.

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