Original article

Oxidant-antioxidant balance in childhood asthma

Background: *Asthmatic patients generate reactive oxygen species impairing the antioxidant defense system and creating a state of oxidative stress in asthmatics.* ***Objectives:*** *Determination of the oxidant - antioxidant status in asthmatic children, by measuring the activities of antioxidant enzymes; superoxide dismutases (SOD) and glutathione peroxidases (Gpx) and estimating plasma level of malondialdehyde (MDA) as an index of lipid peroxidation, to find a relation between antioxidant levels and the severity of asthma and the early response to treatment.* ***Methods:*** *This study included 60 children; group (1): 40 asthmatic children and group (2): 20 apparently healthy children as a control group. The following were measured in all the children; plasma level of (MDA), erythrocytes (SOD) and (Gpx) (in asthmatic children two samples were taken; the first during acute attack and the second after 48 hours of treatment).* ***Results:*** *Significant lower erythrocyte antioxidant enzymes activities and higher malondialdehyde was found in asthmatic children compared to the control group, either before or after receiving treatment. In asthmatics, MDA was significantly decreasing and SOD was significantly increasing with treatment. MDA was significantly higher, while SOD was significantly lower with the severity of asthma either before or after receiving treatment. A significant negative correlation was observed between MDA with both of SOD and Gpx, in acute asthmatic attacks. A significant positive correlation was detected between the activities of SOD and Gpx enzymes.* ***Conclusion:*** *Acute asthma leads to a considerable oxidative stress that is indicated by the high level of malondialdehyde and low level of antioxidant enzymes.*

Keywords: bronchial asthma, superoxide dismutases (SOD), glutathione peroxidases (Gpx), malondialdehyde (MDA), antioxidants.

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INTRODUCTION

Airways are exposed to high level of environmental oxidants and the inflammatory cells infiltrating the airways produce several inflammatory mediators including a range of toxic reactive oxygen species (ROS)[1]. The mechanism by which oxygen radicals cause asthma pathology is oxidation or nitration of proteins, lipids, or DNA to cause dysfunction of these molecules. In addition, physiological antioxidant system is impaired in asthma, possibly because of inflammation. Thus, the imbalance between oxidants and antioxidants that is called oxidant stress is critical to asthma pathogenesis[2].

Malondialdehyde (MDA), is a marker of lipid peroxidation[3]. Oxidative stress may have many effects on airway function, including airway smooth muscle contraction, induction of airway hyper-responsiveness, mucous hyper-secretion, epithelial shedding and vascular exudation. Furthermore, ROS and lipid peroxidation products can influence the inflammatory response at many levels through its impact on signal transduction mechanisms, activation of redox-sensitive transcription factors, and chromatin regulation resulting in pro-inflammatory cytokine and chemokine gene expression and production[4].

Fortunately, the body has its own antioxidant mechanisms, including enzyme systems and non-enzymatic molecules, which help in preventing severe damage from occurring[5].Antioxidant enzymes, such as superoxide dismutases (SOD) and glutathione peroxidases (GPx) play an important role in offering protection to the airways against oxidative stress. Superoxide dismutases convert superoxide to hydrogen peroxide which is eliminated by glutathione peroxidase. SOD gets inactivated by reactive oxygen and nitrogen species. Thus antioxidant defense is impaired in hyperactive

airways. The situation gets further aggravated during acute exacerbations of asthma.

So, the inflammation of the bronchial tree and enhanced systemic oxidative stress is related to an alteration of antioxidant enzyme activities in childhood asthma[6].

This study aimed at determination of the oxidant - antioxidant status in asthmatic children, by measuring the activities of antioxidant enzymes; superoxide dismutases (SOD) and glutathione peroxidases (Gpx) and the plasma level of malondialdehyde (MDA) as an index of lipid peroxidation. It also aimed to find a relation between antioxidant levels and the severity of asthma and the early response to treatment.

METHODS

This is a prospective case/control study carried out over the period (from 15 February 2009 to15 July 2012) at Pediatric and Biochemistry Departments. The study included children; 1-11 years old who were attending Zagazig University Children Hospital or the outpatient clinic. They were divided into two groups; Group (1): 40 known asthmatic cases (24 males and 16 females) who presented to the hospital with acute attacks of asthma. Group (2): 20 healthy children as a control group (12 males and 8 females).

Asthma was diagnosed on clinical grounds according to GINA guidelines[7] and based on history of recurrent or persistent wheezing with or without dyspnea, and improvement on use of β2-agonists[8,9].

Exclusion criteria included patients who had evidence of other concurrent pulmonary or systemic disease or of any upper or lower respiratory tract infection or who had an acute severe exacerbation of asthma or hospitalization within the last week. Patients who were taking vitamin containing tonics were excluded. Infants under one year of age were excluded because of uncertainty of the diagnosis in this age group.

Patients were classified according to the disease severity into three groups: mild, moderate and severe persistent asthma. The severity of asthma was classified according to GINA guidelines 2004[7-9].

For all patients, a full medical history taking and clinical examination were done to diagnose and classify asthma severity[10]. Blood sampling was obtained twice; first at the time of presentation during acute attack, and the second after 48 hours of treatment with β2-agonist {inhaled form for hospitalized cases (19 asthmatic children) and oral form for outpatient cases (11 asthmatic children)} and oral corticosteroids (prednisone 1-2 mg/kg maximum dose 60mg/ day divided into 3 doses).

Methodology

Two milliliters of peripheral venous blood were taken by venipuncture and collected in EDTA containing tubes. Blood samples were centrifuged for 10 minutes at 4000 rpm and then the plasma was aspirated off. The erythrocytes were washed four times with 3 ml of 0.9% NaCl solution, and centrifuged for 10 minutes at 4000 rpm after each wash. The washed centrifuged erythrocytes was then made up to 2.0 ml with cold distilled water, mixed and left to stand at +4˚C for 15 minutes. Samples were stored at -70˚C till time of use. Plasma MDA and erythrocytes SOD and Gpx activities were measured spectrophotometrically according to the methods of Ohishi et al.[11], Wang et al.[12] and Paglia et al.[13].

Statistical analysis

Data were expressed as (mean±SD) for quantitative variables, numbers and percentage for qualitative variables. The comparison between groups was performed with one way analysis of variance (ANOVA): f test, t test, paired t test, chi-squared (χ^2) or Fisher exact test when appropriate. Correlations were assessed by Pearson's test (r). P-value of less than 0.05 was considered statistically significant.

RESULTS

SOD and Gpx were significantly lower in asthmatics than the control group. Lower SOD level was noticed before than after treatment. MDA was significantly higher in asthmatics, and its level was higher during the acute attack before treatment (table 2). Before treatment, SOD showed lower levels in moderate and severe asthma than mild asthma. Gpx showed no difference between mild, moderate and severe asthmatics. MDA was significantly higher in severe asthma, than moderate asthma. Mild ones showed the lowest levels (table 3). After treatment SOD and Gpx showed no difference among mild, moderate and severe asthmatics. MDA showed higher levels in severe asthmatics than mild and moderate ones (table 4). There was a significant positive correlation between SOD and Gpx in asthmatics before treatment, (figure 1). Gpx and MDA correlated negatively both before and after treatment [r -0.057, -0.29, p <0.001, <0.05 respectively] (figure 2).

Table 1. Demographic data of the studied cases.

History (N = 40)	***N***	***(%)***
Residence:		
Urban	23	57.5
Rural	15	37.5
Housing:		
good aeration	28	70
Sun exposure of carpets	27	67.5
House hold pets	18	45
Family history of atopy	20	50
Consanguinity	17	42.5
Parental smoking	22	57.5
Lactation:		
breast milk only	15	37.5
Introduction of cow milk in the first year	25	62.5
Good intake of fresh fruits & vegetables	17	42.5
Food allergy	9	22.5
Atopy other than asthma	13	32.5
Precipitating factors:		
dust	28	70
Viral infection	27	67.5
Smoke	17	42.5
Animals &birds	14	35
Chalk dust	1	2.5
Fumes	5	12.5

Table 2. Anti-oxidant enzyme activities and malondialdehyde levels in asthmatics (before and after treatment) and in the control group.

parameter	**Cases n=40 before treatment**	**Cases n=40 after treatment**	**Control n=20**	**F**	**P**
SOD	23.17 ± 2.1	25.9 ± 2.4	28.7 ± 3.1	6.4	<0.001
Gpx	47.86 ± 3.1	48.2 ± 2.9	54.5 ± 4.9	1.07	>0.05
MDA	19.8 ± 6.6	11.1 ± 5.2	7.6 ± 2.3	7.82	<0.001

SOD: super oxide dismutase, Gpx: glutathione peroxidases, MDA: Malondialdehyde, p<0.05 significant

Table 3. Pre-treatment antioxidant enzyme activities and malondialdehyde levels in different degrees of asthma severity.

Parameter	**Severity**			**F**	**P**
	Mild	**Moderate**	**Severe**		
SOD	24.2 ± 1.2	22.9 ± 2.4	22.1 ± 2.1	3.1	<0.05
Gpx	48.4 ± 3.6	48.6 ± 2.8	45.8 ±2.2	2.8	>0.05
MDA	13.1 ± 2.6	20.1 ± 2.8	28.5 ± 3.7	75.0	<0.001

SOD: super oxide dismutase, Gpx: glutathione peroxidases, MDA: Malondialdehyde, p<0.05 significant

Table 4. Post-treatment antioxidant enzyme activities and malondialdehyde levels in different degrees of asthma severity.

Parameter	**Severity**			**F**	**P**
	Mild	**Moderate**	**Severe**		
SOD	27 ± 2.9	25.1 ± 1.9	25.5 ± 1.8	2.73	>0.05
Gpx	48.8 ± 3.4	48.8 ± 2.7	46.2 ± 1.9	3.03	>0.05
MDA	9.1 ± 3.8	10.3 ± 4.6	15.1 ± 6.3	4.5	<0.05

SOD: super oxide dismutase, Gpx: glutathione peroxidases, MDA: Malondialdehyde, p<0.05 significant

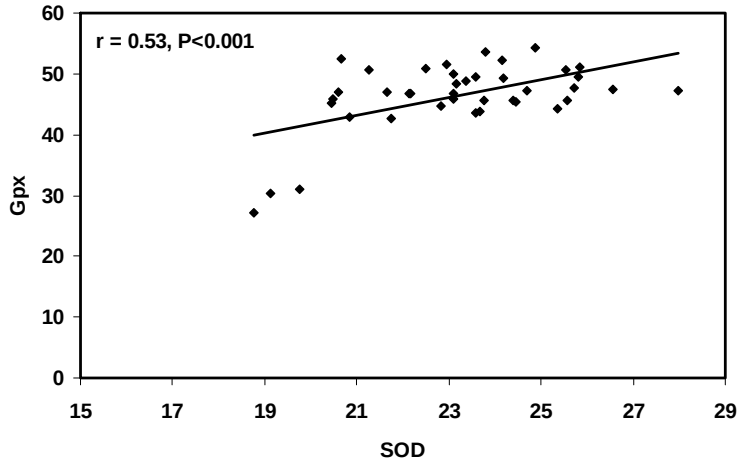


Figure 1. Correlation between SOD and Gpx in cases before treatment.

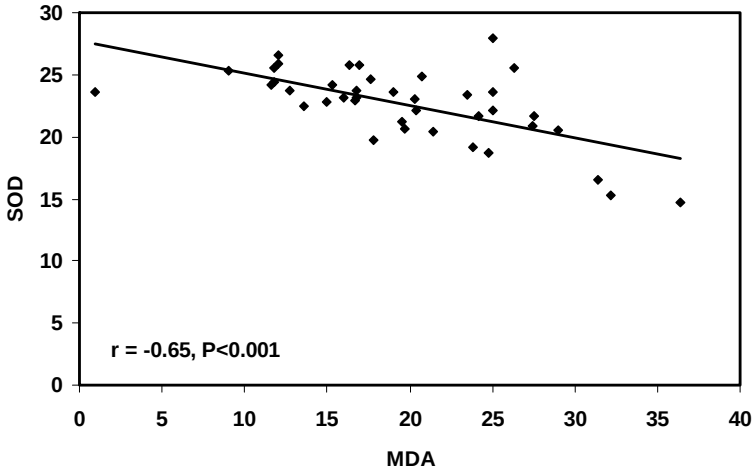
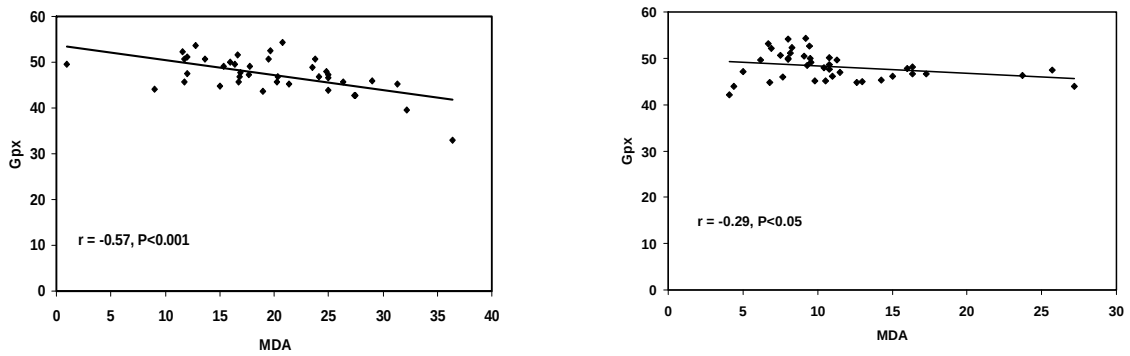


Figure 2. Correlation between SOD and MDA in cases before treatment.



(a) ***(b)***

Figure 3. Correlation between Gpx and MDA in cases (a) before and (b) after treatment.

DISCUSSION

Asthma itself may cause physiological changes in serum antioxidant status, perhaps because of increased oxidant burden associated with the disease. Numerous disturbances of antioxidant defense mechanisms have been described in asthma[14]. The situation gets further aggravated during acute exacerbations of asthma[15].

Our study demonstrated a significant decrease in erythrocyte SOD and Gpx enzymes activities in asthmatic children; either during acute attack or after 48 hours of treatment.

This finding was consistent with other observations[4-6-16-17-18]. In those studies, one sample was taken from the patient to test the antioxidant enzyme activity. In our study, we evaluated the antioxidant enzymes twice to detect the effect of treatment. We found significant lower activity of erythrocyte SOD enzyme but not Gpx enzyme before than after treatment.

A parallel study documented loss of reducing potential by non-enzymatic and enzymatic antioxidants in asthma as compared to healthy individuals, with lowest levels in those patients with the most severe asthma[14].

In our study we found that SOD enzyme activity during the acute attack of asthma was affected by disease severity; where it was significantly higher in mild than moderate and severe asthmatics. This result was consistent with other studies[6-19-20]. After 48 hours of treatment, the SOD activity became comparable in different grades of asthma.

Gpx enzyme activity was not affected by the severity of asthma both before and after 48 hours of treatment. A similar result was observed by Mak et al.[21]. On the other hand, Al-Faleg et al.[22] found that there was no significant correlation between severity of asthma and the various measured SOD and Gpx activities.

In consistence with many other observations[17-22-23-24] our study revealed a significant elevation of MDA plasma level among the asthmatic children both during the acute attack and after 48 hours of treatment compared to control group with the level being significantly higher before than after treatment.

We also found that plasma level of MDA during the acute attack was related to severity. MDA level was significantly higher in severe than moderate and mild cases, both before and after treatment.

So in general, asthma severity could be related to the extent of lipid peroxidation, with a positive association between MDA concentration and disease severity. This was similar to the findings of other studies[25-26].

The documented decrease in plasma level of MDA after 48 hours of treatment in our results is also supported by the findings of Sharma et al.[3]. They reported that serum MDA levels were highest at the time of admission, and decreased significantly at 24–48 hours with treatment. After a 3 weeks symptom free interval serum MDA levels had decreased further but remained higher than those of healthy control group. So, they concluded that lipid peroxidation is increased in bronchial asthma during an acute attack and symptom free period as well[3].

The increase in reactive oxygen species (ROS) during asthmatic attack, as demonstrated indirectly by significant elevation of MDA, might overwhelm endogenous antioxidant defenses. This finding was illustrated in the present work by the low levels of two antioxidant enzymes SOD and Gpx and it is in agreement with the observations of other researchers[25-26].

In our cases, we found a significant negative correlation between plasma level of MDA and both of erythrocyte SOD and Gpx enzymes activities during the acute attack. This was in agreement with the observations of other researchers[14]. After treatment, the negative correlation was detected only between MDA and Gpx.

In conclusion, asthmatic patients suffer from an oxidative stress as indicated by the high level of the oxidant MDA and low level of the antioxidant enzymes SOD and Gpx. This oxidant-antioxidant imbalance is related to the severity of asthma, especially during the acute attack and is more evident before treatment. The possibility of the introduction of antioxidants as a line of treatment of asthma needs further studies.

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