Evaluation of oxidative stress in bronchio-asthmatic children in Qassim

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Abstract: Bronchial asthma is one of very common diseases in Saudi Arabia. Exposing the body to high oxidizing agent daily during the work will cause a failure of the defense system (immunity system) to protect of the body, which increase the number of oxidizing agent in the body which known as vintage (oxidative stress) which consider as mean factor risk for many diseases one of them is Bronchial asthma. This work aims to investigate oxidative status in asthmatic patients in Qassim region as a measure of oxidative stress that may affect hematological parameters. Oxidant/antioxidant parameters were estimated in blood samples such as; hydrogen peroxide, Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Malondialdehyde, The total protein, carbonyl protein and blood indices. The results of the biochemical parameters of patients were compared to that of the normal control group. The results showed that samples of asthmatics group had high rates of oxidative stress, accompanied by a major imbalance in the amount of antioxidants. In addition, high levels of lipid peroxidation products and carbonyl protein, was also associated with a reduction in the rates of total protein and blood content of hemoglobin. The results also showed a significant decline in the blood indices as well as blood cell count as compared to the control group. In conclusion, the study reveals a critical imbalance in oxidant/antioxidant status accompanied by a decline in hematological parameters that may lead to permanent inflammation if not diagnosed early and treated.
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Key wards: bronchial asthma- oxidant- antioxidant- blood indices.

1. Introduction

Asthma continues to cause considerable disability in children and adults throughout the world. It is the most common chronic disease of childhood and an important cause of absence from school and reduced participation in sports and other activities (Hill *et al.,* 1989a). Several studies, for example in the UK and Australia, have reported a substantial increase in the prevalence of the disease among children over the last three decades (Peat *et al.,*1992 & Omran & Russell, 1996). While some of this may be due to changes in diagnostic practice, there is general agreement that the increase in prevalence of symptoms is a true phenomenon (Hill *et al.,*1989b & Burney *et al.,*1990). The change seems to be associated with changes in lifestyle (Shaheen,1995 & Woolcock,1996) and this is supported by studies which have shown an increased prevalence of asthmatic symptoms among people who have moved from a traditional to a more Westernized style of living (Van Niekerk *et al.,*1979 & Flynn,1994).

The disease is now reaching epidemic proportions in Westernized countries, recent studies having reported prevalences of diagnosed asthma among primary schoolchildren in urban Aberdeen, Scotland, of almost 20% and of exercise-induced bronchospasm in the rural Isle of Skye as high as 30% (Austin *et al.,*1994 & Omran & Russell,1996). The causes of the increase in asthma, which appears to have been accompanied by smaller rises in the prevalence of hay fever and eczema, remain unknown. These recent results suggest that urbanization *per se* is not responsible and one of the present authors has argued that increased population susceptibility rather than changes in exposure to allergens is likely to be the explanation (Seaton *et al.,*1994 & Seaton *et al.,* 1996). The association with a Westernized lifestyle appears to be strong, and changes in diet and patterns of childhood infection at present seem to have been the most likely determinants of the proposed changes in population susceptibility (Seaton *et al.,*1994; Shaheen,1995; Hodge *et al.,*1996 & Soutar *et al.,* 1997).

Kingdom of Saudi Arabia (KSA) is a country that has developed rapidly over the last three decades as a consequence of the importance of oil to the world's economy, also it has moved from a primarily rural to a wealthy urban economy. Most people in the population live in large modern cities, in a style far removed from that of their forefathers. Nevertheless, in country districts and villages a much more traditional lifestyle is maintained. Previous studies have suggested that doctor-diagnosed asthma occurs in 4-17% of urban Saudi children (Al Frayh,1990a; Al Frayh *et al.,*1992 & Hijazi *et al.,*1998). In contrast, the change in the environment directly or indirectly could be responsible for the observed increase in the prevalence of asthma (Al Frayh *et al.,*2001).

The environmental pollution by aeroallergens may be responsible for the rising asthma prevalence

in the KSA. This is supported by the fact that there was a 5% rise in the number of allergic rhinitis sufferers as well. Increased prevalence of asthma and allergic rhinitis attributable to environmental pollution has also been reported in other studies (Peat *et al.,* 1994 & Hopper *et al.,*1995).

Exposure to higher allergen levels both outdoors and indoors may have increased airway abnormalities in the Saudi children. The soil and climate of the KSA was once considered unfavorable for plant growth. A large number of plants have been introduced to the Kingdom in recent years. Fungal spores and airborne pollens of grasses, weeds, and trees have been detected using a Burkard trap (Burkard Manufacturing Co., Rickmansworth, Hertfordshire, England) in the KSA (Al Nahdi *et al.,* 1989).

A high proportion of Saudi children suffering from asthma have been shown to react to the extracts of these aeroallergens on skin prick testing. Among outdoor allergens, grass pollens and weed extracts have been shown to be the most common allergens to cause a reaction to the skin prick testing (Al Frayh, 1990b). It is, therefore, not inconceivable that introduction of some new outdoor eroallergens by imported plants and trees may have contributed to the increased prevalence of asthma in the KSA. In Riyadh and other cities in KSA, it was shown that 63.7% of individuals react to one or more allergens of indoor origin (Al Frayh *et al.,*1990).

Increased generation of oxidants has been reported in asthma which aggravated airway inflammation by inducing diverse pro-inflammatory mediators including macrophages, Neutrophiles and Eosinophiles (Rahman *et al.,*1996 & Terada, 2006). Several studies have suggested that oxidative stress is caused by overproduction of different free radicals or by an insufficient antioxidant defense system in asthma which is induced by inflammatory cells. The oxidant–antioxidant status was investigated in blood because it is an available source and also considered as an important pool of antioxidant defenses in the body (Kirkham & Rahman, 2006).

This work aims to investigate oxidative status in asthmatic patients as a measure of oxidative stress that may affect hematological parameters.

2.Material and Methods

Experimental Design:

Fourty girls and boys aged between (8-13) years attending the outpatients clinic in General Buraydah hospital were enrolled in this study and divided into two groups:

Group I: Include 20 healthy children as control normal group

Group II: Include 20 children with bronchial asthma.

Five milliliters of blood were withdrawn from each individual and divided into three parts.

The first part

Was collected in lithium heparin tubes for the following determinations:

- Quantitative determination of glutathione peroxidase.
- Quantitative determination of superoxide dismutase.
- Quantitative determination of hemoglobin.

The second part

Was collected in lithium heparin tubes and centrifuge at benchtop for 15 min for separated plasma and for the following determinations:

- Quantitative determination of total protein.
- Quantitative determination of carbonyl protein.
- Quantitative determination of malondialdehyde.

The third part

Was collected in normal tubes and centrifuge for 15 min to separate serum to investigate hydrogen peroxide.

Biochemical Parameters:

Serum samples were assayed for total protein according to Josephson & Gyllensward,1957, carbonyl protein according to Levine *et al.,*1990, malondialdehyde according to Vento *et al.,*2000, and hydrogen peroxide according to Mc Namara, & Augusteyn,1984, Whole blood samples were assayed for blood indices according to Van Kampen & Zijlstra,1961, glutathione peroxidase according to Kraus & Ganther,1980, and super oxide dismutase according to Suttle, 1986.

Statistical Analysis:

The data analysis was carried out using the statistical package for social science (SPSS software version 16, Chicago, Illinois). All numeric values were expressed as mean ± SE. Statistical comparisons were performed using independent sample's T test. Pearson's correlation test was used for correlating variables. For all tests a probability value less than 0.01 was considered significant.

3.Results

Data of the present study revealed a state of airway inflammation and bronchial asthma as represented in table (1) and figure (1).

Table (1): Oxidant/antioxidant status in the studied groups.

	Control	Patients
Hydrogen peroxide (µM)	128.9 ±0.22	143.9 ± 0.16*
Malodialdehyde (µ/M)	3.9 ± 0.32	11.4 ± 0.42*
Total protein (g/dl)	72.4 ± 0.19	67.2 ±0.34
Carbonyl protein (nmol/mg)	1.15 ±0.007	1.39 ±0.012
Glutathione peroxidase (U/gm Hb)	160.2 ± 1.74	126.3 ±0.87*
Superoxide dismutase (U/ml)	451.3 ±5.4	336.7 ± 5.9*

Data are represented as mean ± SE. *$P < 0.01$

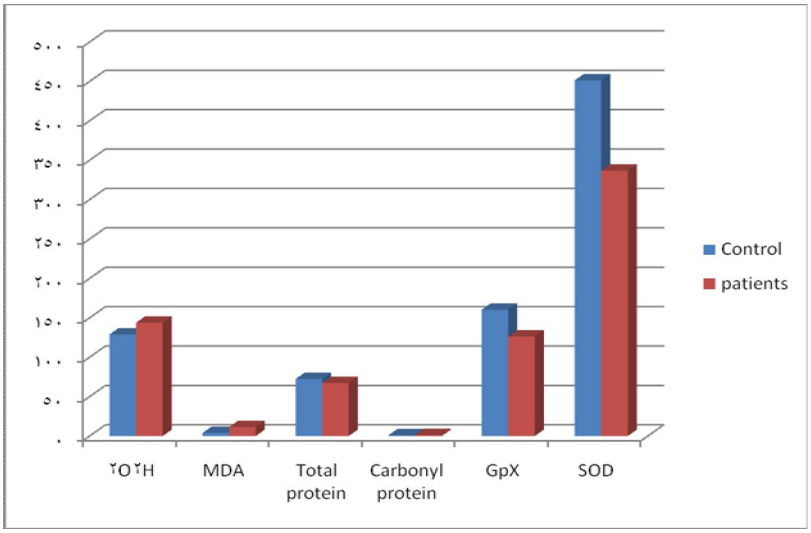

Figure (1): Comparison of the oxidant/antioxidant status in the studied groups

Table (2): Blood indices in the studied groups.

	Control	Patients
Hemoglobin (g/dl)	13.8 ± 0.09	12.4 ± 0.08
(%)Hematocrit	41.2 ± 0.25	37.4 ± 0.23*
Mean cell volume (f/L)	80.4 ± 0.14	77.1 ± 0.38*
Red Blood Cell Count (10^6/uL)	5.1 ± 0.03	4.8 ± 0.03
White Blood Cell Count (10^3/uL)	8.9 ± 0.15	6.7 ± 0.08 *

Data are represented as mean ± SE. *$P < 0.01$

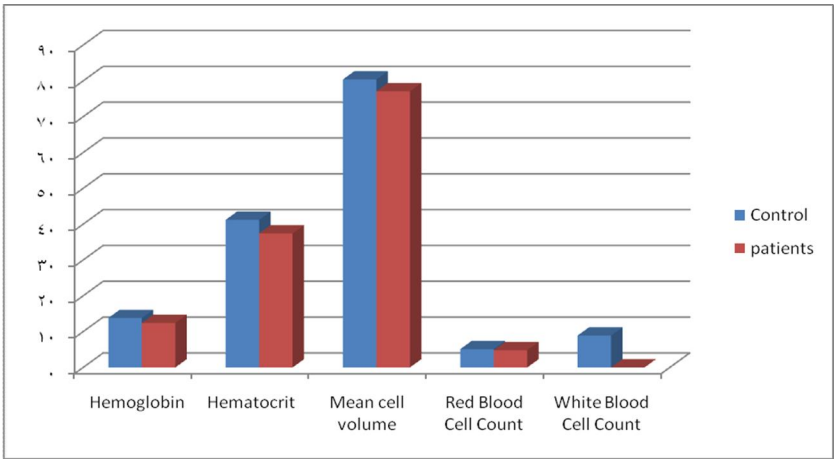

Figure (2): Comparison of blood indices in the studied groups.

Symptoms of bronchial asthma has been proved biochemically in asthmatic group by the statistical significant increase in malondialdehyde, carbonyl group and hydrogen peroxide serum levels as compared to their normal counter parts ($P < 0.01$).

Moreover, such result was confirmed by a statistical decrease in blood indices, whole blood superoxide dismutase activity and glutathione peroxidase activity ($P < 0.01$) as shown in table (2) and figure (2). However, the decrease in the total protein level did not reach a statistical significant level between the same comparative groups.

The correlation between the investigated oxidant and antioxidant status using Pearson´s correlation is represented in table (3). It reveals a highly statistically significant negative correlation between antioxidant parameters represented by superoxide dismutase and glutathione peroxidase as well as hemoglobin with the evaluated oxidant parameters ($p < 0.001$). Besides, a highly statistically significant positive correlation within the studied oxidants was also shown. Scatter plot diagrams of these data are represented in figure (3).

Smokers in the family and number of cigarettes smoked in the house have also been shown to be associated with childhood asthma (**Al Frayh,1990a**). A significant increase in the smoking habit of the family members of the children with asthma was observed in this study. This may reflect a general increase in the prevalence of smoking in the Saudi population. It is therefore also possible that cigarette smoke may have contributed to an increase in the prevalence of asthma by irritating asthmatic children's airways, already inflamed by exposure to various allergens.

Exposure to different stimuli results in the generation of reactive oxygen species (ROS) in the airway epithelial cells, which produce inflammatory cytokines and chemokines and express adhesion molecules on their cell surface and cause airway inflammation, which involves narrowing of airways, secretion of large amounts of mucus, and infiltration of inflammatory cells (Holgate,2008). Increasing evidences suggest that ROS play an important role in the pathogenesis of airway inflammation during asthma (Sheppard,2009).

4.Discussion

Table (2): Pearson´s correlation coefficient in the studied groups

	H_2O_2 (µM)	MDA (µM)	SOD (U/ml)	Gpx (U/gm Hb)	Hb (g/dl)	Ht (%)	MCV (f/L)	RBC (10^6/ uL)	C.P (nmol/mg)	T.P (g/dl)
WBC (10^3/uL)	- 0.90 **	- 0.81 **	0.87 **	0.93 **	0.88 **	0.87 **	0.64 **	0.75 **	- 0.86 **	0.83 **
H_2O_2 (µM)	-------	0.93 **	-0.91 **	-0.95 **	-0.90 **	-0.86 **	-0.78 **	-0.61 **	0.94 **	-0.91 **
MDA (µM)		-------	-0.83 **	-0.91 **	-0.87 **	-0.80 **	-0.78 **	-0.51 **	0.82 **	-0.76 **
SOD (U/ml)			-------	-0.83 **	0.85 **	0.88 **	0.70 **	0.70 **	-0.85 **	0.90 **
Gpx (U/gm Hb)				-------	0.93 **	0.88 **	0.80 **	0.58 **	-0.90 **	0.82 **
Hb (g/dl)					------	0.89 **	0.85 **	0.61 **	-0.82 **	0.76 **

T.P: total protein; C.P: carbonyl protein. $^{**}P < 0.001$

ROS play an important role in the pathogenesis of airway inflammation during asthma by disturbing the cellular redox homeostasis.

With growing understanding of the role of ROS in mediating the airway inflammation, various studies have suggested the use of antioxidants to treat such inflammation (Kirkham & Rahman,2006). Although the antioxidant capacity of airway epithelial cells is excellent, upon repeated and continued exposure to allergens, the antioxidant capacity decreases. This further augments the ROS generation and inflammation. Therefore, antioxidant(s) or the compounds that could block the inflammatory signals and/or the transcription of inflammatory markers could be excellent drugs to treat airway inflammation. Increased dietary intake of ascorbic acid has been shown to improve lung function in asthma patients (Yadav *et al.,*2009).

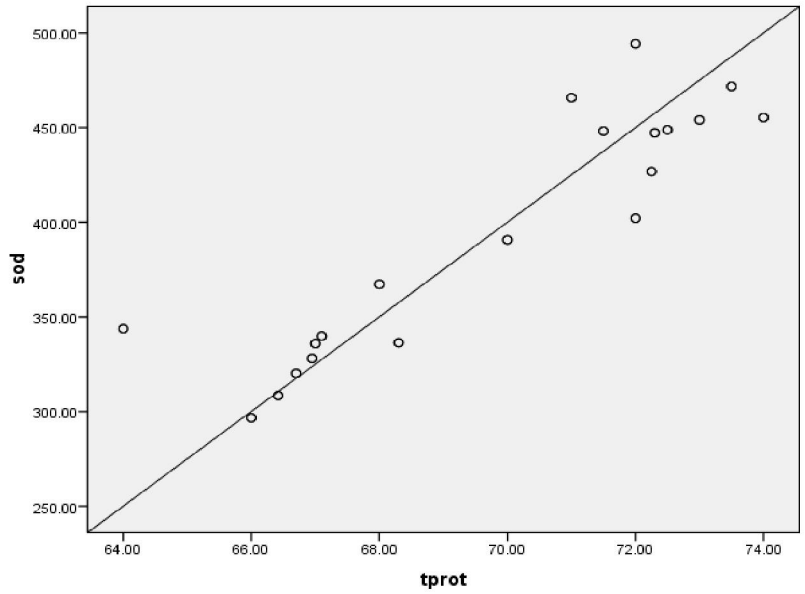

Figure (3): Scatter plot of the correlation between superoxide dismutase and total protein

In this study, we demonstrated the presence of early airway hyper responsiveness or bronchoconstrictive responses revealed by a significant increase in the oxidant parameters and a significant decrease in the antioxidants when compared to their normal counterparts.

Rahman *et al.* (1996) reported that the plasma MDA level was increased in asthmatic patients as well as in patients with asthma exacerbation as compared to stable asthma. Similarly, another study entails that MDA level in bronchoalveolar ravage (BAL) fluid was higher in mild to moderate asthmatic patients. Moreover, protein carbonyl content was also significantly higher in asthmatic patients because most of the amino acids can be oxidized by ROS (Ozarus *et al.,*2000). Peroxynitrite anion is a strong oxidant that mediates not only the oxidation of both non-protein and protein sulfhydryls but also induces lipid peroxidation.

The alterations in antioxidant defenses may involve either an increase or a decrease depending on the changes occurring due to a defense response. Glutathione peroxidase (GPx) plays a significant role in peroxyl scavenging mechanism and maintaining functional integration of the cell membrane. Lower GPx level in asthmatic patients can be related to the clinical presentation of the disease and it indicates the presence of H_2O_2 in breath condensate of exhaled air which is elevated in asthmatics patients. Selenium is an essential component of GPx and it indirectly helps in protecting cells against damage caused by free radicals. This might arise as a result of deficiency of selenium or inactivation caused by $OH^{\cdot}$ and $O_2^{\cdot -}$. A number of studies have been done to measure the selenium deficiency in asthma through the antioxidant effects of GPx and it has been found that plasma levels of selenium was significantly lower in asthmatic patients (Pearson *et al.,*2004).

Glutathione peroxidase (GPx) is essential for removing toxic lipid oxidation products and H_2O_2, which are continuously generated as a result of sequestration and infiltration of inflammatory leukocytes in the lung. Low superoxide dismutase (SOD) activity together with low GPx activity in asthmatic patients proved the contribution of oxidative stress in the etiology of asthma. It was reported that the glutathione system is altered in lung inflammatory conditions such as asthma and many reports have shown that alterations in glutathione level have been found in asthmatic airways (Smith *et al.,*1993).

Mak *et al.* (2004) have also reported that the total glutathione level increased in erythrocytes of asthmatic individuals. There have been several reports on the decreased antioxidant capacity in asthmatic patients with intense oxidative load.

Al-Abdulla *et al.* (2010) also reported that the mean serum level of MDA was significantly raised with increasing severity of asthmatic attack among patients grouped according to degree of severity.

Some workers also demonstrated that the superoxide anion release was greater in patients with exacerbation of their disease and it was also inversely correlated with FEV1 (Comhair *et al.,*2000).

Oxidative stress is involved in asthma. Carbonylated proteins (68 kDa and 53 kDa) were elevated in asthmatics when compared to controls and the 68-kDa carbonylated protein was

significantly correlated with sputum eosinophilia (Nagai *et al.,* 2008).

In the present work, we have shown that carbonyl group level was elevated in bronchial asthma induced group. Similar findings were observed in mice after chronic exposure to ozone for 6 weeks. Antibodies against carbonyl-modified protein were elevated and splenocytes isolated from ozone-exposed mice became activated in response to stimulation with carbonyl-modified protein. This was accompanied by a greater antigen-presenting cell activation (both macrophages and dendritic cells) in murine lungs as demonstrated by the increased expression of the activation markers CD80, CD86, and CD54 on these cells (Kirkham, *et al.,*2011).

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