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Role of Antioxidants and Antioxidant Enzymes in Asthmatic Children

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ABSTRACT
In children, respiratory system is not completely developed .Continuous exposure to environmental factors, pollutants and various infectious agents such as viruses, bacteria etc, makes the system vulnerable to inflammatory reactions. Epithelial cells, resident macrophages, endothelial cells and recruited inflammatory cells, generate ROS in response to increased levels of secretory toxins. ROS produced by phagocytes at sites of inflammation and increased oxidative stress are a major cause of cell and tissue damage associated with various acute and chronic inflammatory lung diseases.
Clinically diagnosed and established seventy five cases of Asthma with sixty normal healthy controls in paediatric group were included in this study. Efficacy of the antioxidant system was evaluated by measuring specific activities of antioxidant enzymes PON1, SOD, CAT, GPx, GRx and measuring antioxidants vitamin E and ascorbic acid .Present study shows significant decrease in antioxidant enzymes SOD, CAT, PON-1 ($p<0.001$) and antioxidants with increased levels of MDA ($p<0.001$) with moderate changes in GPx and GRx levels in asthmatic children as compared to control.
Keywords- *ROS, antioxidants, paraoxonase1, asthma.*

Introduction

Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism. At low to moderate concentrations, they are essential for physiological cell processes, but at high concentrations, they produce adverse modifications in cell components, such as lipids, proteins, DNA etc. Globally extensive basic research to define more clearly the role of free radicals and oxidative stress in pathological conditions is being carried out. According to Knight et al[1] continuing clinical research will lead to more reliable preventive measures and treatment modalities for them.

Respiratory system

In childhood, lung development takes place in the age of first 6 – 8 years so pulmonary susceptibility to infection is mostly related to immature lung tissue, innate immunity and age specific respiratory pathogens. Chronic exposure of lungs to viral, bacterial or parasite infections results in infectious

and inflammatory conditions leading to disruption in the functions of respiratory system, termed as acute respiratory failure. To fight against these infections, neutrophils and other phagocytes attack pathogens by producing no of reactive oxygen species: singlet oxygen (O_2^-), nitric oxide (NO), hydrogen peroxide (H_2O_2), hypochlorous acid. These free radicals or reactive oxygen species gives rise to oxidation of membrane PUFA. It is represented with common clinical signs such as respiratory tachypnea, altered depth and pattern of respiration, tachycardia, restlessness, headache, irritability, and wheezing and in untreated cases results into cyanosis, seizures, cardiac arrest or coma. The commonest laboratory findings are hypoxemia, hypercapnia & acidosis. Finkelstein et al [2] observed adverse long-term consequences in lung development in untreated paediatric patients with respiratory infections. Behraman et al [3]emphasized that early evaluation and management in the predisposed infants and school going children is necessary to avoid high morbidity in the adult life.

Asthma

Asthma is one of the major noncommunicable, chronic inflammatory conditions of the lung airways resulting into episodic airflow obstruction. Wheezing, shortness of breath, chest tightness are the most common symptoms of asthma in children. WHO estimates that 235 million people currently suffer from asthma.20% of all children have had at least one wheezing illness by 1 year of age, almost 33% by 3 year of age and nearly 50% by 6 year of age[4]. Physical exertion, airways irritants and infection are some triggering factors for broncho constriction of the bronchiolar muscular bands. A cellular inflammatory infiltrate of eosinophils, mast cells, lymphocytes release pro-allergic, pro-inflammatory cytokines and chemokines fill up the airways. This leads to epithelial damage and desquamation of the airways lumen, affecting lung function. Considering all these metabolic events, present study was undertaken to evaluate the role of oxidative stress and to study imbalance in oxidants/antioxidants, and its correlation to pathogenesis of asthma.

Material and Methods

Present study was conducted in the department of Biochemistry, and tertiary care hospital after taking college ethical committee approval.

Study group comprised of clinically diagnosed and established seventy five asthmatic children with sixty normal healthy controls in paediatric group. All children selected in this study were untreated new cases of asthma in the age group of six months to twelve years of both the sex and attending paediatric OPD and IPD, of tertiary care Hospital.

The diagnosis was based on the signs and symptoms, as well as in some cases depending on X-ray chest and hemogram.

Collection of blood sample

3mL venous blood was drawn from each subject with prior written consent from parents/guardian. The collected blood was divided into two parts, 2 ml in EDTA and 1ml in plain vacutainer aseptically. The first part was further seperated into plasma and erythrocytes by centrifugation at 3200- 3500rpm for 15 min by taking necessary precautions to avoid haemolysis. Plasma was used for the determination of lipid peroxidation (LP) in terms of MDA levels [5], vitamin E [6] vitamin C[7]. Erythrocytes were washed with 0.9% saline for three times. Then cold distilled water was added to haemolyse RBC. This hemolysate was used for the determination of superoxide dismutase (SOD) [8] glutathione peroxidase (GSH-Px)[9] glutathione reductase (GSH-Rx)[10] and catalase (CAT) [11]. The second part of the sample was centrifuged and serum was separated. Serum sample was used for determination of paraoxonase (PON1) [12].

All the methods were standardised on Perkin Elmer UV-VIS spectrophotometer by using in house regents or by commercial kits available.

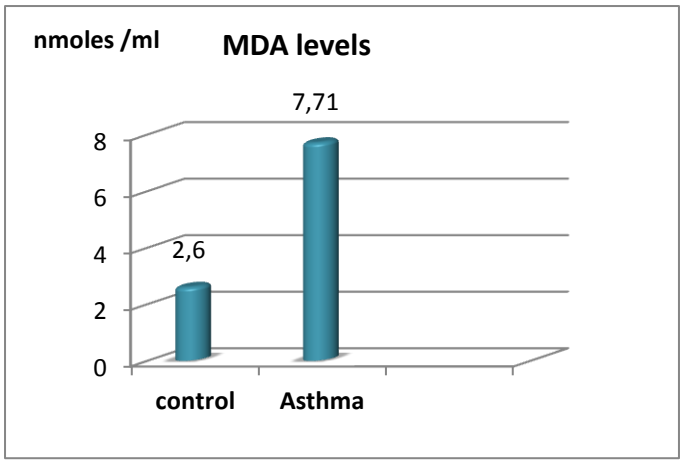
Tables and Results

All statistical analyses were carried out using the SPSS (statistical package for the social science

software) statistical package Windows, and the software Microsoft Excel. Quantitative data were expressed as mean and standard deviation(X +SD) and analyzed by applying Student's t-test for comparison of two groups of normally distributed variables. The results of the ''t''-value are then checked on Student's ''t''-table to find out the significance level (p-value) according to the degree of freedom. All these tests were used as tests of significance at p <0.01

A total of one hundred thirty five subjects, including seventy five asthma patients, and sixty healthy children as controls were studied. The mean age of the asthma patients was 3.3± 0.9years and male/female ratio was 47/28 while in control group, mean age of the children was 4.2± 0.6years, and male/female ratio was 39/21.

Graph 1: Lipid peroxidation in terms of plasma MDA Levels in control and in asthma



P<0.01

Table 1: Plasma levels of antioxidants vitamin C, and Vitamin E with control as well as within the study groups

Groups	Control n(60)	Asthma n(75)
Parameters	**Mean ±SD**	**Mean ±SD**
Vit C mg/dl	2.70±0.51	1.25±0.20
Vit E mg/dl	2.34±0.38	1.76±0.46

p<0.01

Table 2: Specific activities of SOD, CAT, PON1, GPx, GRx antioxidant enzymes in control and asthma subjects

Groups	Control n(60)	Asthma n(75)
Parameters	**Mean ±SD**	**Mean ±SD**
SOD U/mg of Hb	3.81±0.49	2.33±0.28
Catalase K/mg of Hb	347.31±51.20	256.16±24.34
PON1 IU/L	232.54±34.69	136.57±13.54
GPX U/g of Hb	67.90±12.37	64.9±8.69*
GRx U/g of Hb	12.05±2.57	11.91±2.46*

p<0.01 *p>0.5

Discussions

In present study MDA levels were found significantly increased in asthmatic children as compared to control group .This was suggestive of increased lipid peroxidation in asthma when compared to control group.

This increased lipid peroxidation which could have triggered inflammatory pathway of cytokines and chemokines. This might have attracted phagocytes, granulocytes, eosinophils and mast cells at the site of alveolar tissue. This increased inflammatory reactions and increase in ROS are suggestive of the observed pulmonary tissue damage which induce symptoms like bronchoconstriction, elevated mucus secretion, and cause micro vascular leakage, which leads to edema formation and pathogenesis in asthma cases. Shokry et al [13] in his study observed similar results with significantly higher, MDA levels while significant negative correlation of MDA with both of SOD and GPx, in acute asthmatic attacks.

In this study, levels of antioxidant enzymes SOD, CAT, PON1 activity were observed statistically decreased when compared to controls. GPx and GRx activity was observed as moderately decreased but statistically significant difference (p>0.5) was not observed.

Comhair et al [14] observed reduced SOD activity in the oxidant-rich environment of the asthmatic airways and during asthma exacerbation. Further loss of SOD activity observed was with enhanced production of oxygen radicals by inflammatory cells which would be reflected systemically in loss of circulating SOD activity and clinically by development of severe asthma and/or worsening airflow limitation.

Mate´s et al [15] observed enhanced, SODs and CAT activities and a decrease in GPx activity in mononuclear cells from allergic patients compared to controls. Conversely, in erythrocytes, he observed higher values for GPx and SODs and similar CAT activities were found in allergic patients and controls.

In physiological conditions GPX/GRx enzyme system continuously replenishes reduced glutathione in lung tissue to scavenge the increased load of ROS and lipid peroxidation with the help of various antioxidants like vitamin C, vitamin E and PUFAs.In our study, in asthmatic children, GPx and GRx values were not significantly changed as compared to control group which might be suggestive of sustained activity of GPx/GRx for counterbalancing lipid peroxidation. Igor et al [16] observed increased expression of GRx and GPx in their in vitro studies on rat liver. They observed that microsomal membrane lipid peroxidation leads to calcium release and uncontrolled activation of calcium-dependent proteases and lipases whereas ROS attack on mitochondrial membranes alters permeability and induce a disruption of cellular energetics. In addition, an accumulation of lipid peroxidation products under pathological conditions indicated the involvement of oxygen radicals in these disorders. The results of his study indicated that induction of synthesis of these enzymes may be one of the factors promoting increased activity of the GRx/GPx system and over expression of these enzymes was found to be important for increased AREs function which is associated with GR gene expression, and resistance to oxidative stress, the key pathogenic factor in various diseases.

Clara cells are one of the oxidant resistant airway cells in lungs of all species. PON1 is mainly localized in clara cells, endothelial cells and in type 1 pneumocytes in the lungs.

In the present study significantly lowered activity of PON 1 was observed in asthma patients as compared to control group. This could be due to shedding off and continuous replacement of clara cells by mucosal cells due to increased lipid peroxidation and increased oxidative stress [17].

Vitamin E is a lipid soluble antioxidant that represents the principal defence against oxidant induced membrane injury and protects membrane PUFAs.

In present study, we observed antioxidants vitamin C, vitamin E levels were found significantly decreased (p value-<0.01) when compared to control group which were suggestive of maximum utilization of antioxidants in counterattacking ROS molecules during lipid peroxidation in disease progress.

Similar observations were noted by Oluwole et al [18] in their study on exposure to household pollution from biomass fuel and its association with pulmonary dysfunction, reduced antioxidant defence and inflammation of the airways. They observed that exposure to pollutants increased production of free radicals and inflammation which exceeded the capacity of the antioxidant defence system, resulted in increased lipid peroxidation and decreased SOD, vitamin C and vitamin E

Birben et al [19] observed significantly lowered serum levels of antioxidant compounds like vitamin C, vitamin E and significantly high MDA levels as compared with the controls. These levels very well correlated with increasing severity of asthmatic attack. He observed significant negative correlation between MDA with vitamin C during acute asthmatic attacks .Similar results were also noted in children with pneumonia[20]

Antioxidants vitamin C and vitamin E supplementation and nasal inflammatory responses among young asthmatics exposed to high levels of ozone were studied by Sienra-Monge et al [21]. They observed antioxidant supplementations of vitamin C

and vitamin E to some extent provides protection against asthma with lowered glutathione levels.

Ehrenkranz et al [22] could not observe in their randomised controlled trials using prophylactic vitamin E supplementation, preventive role of vitamin E in chronic lung diseases. Similar studies were done by Kelly et al [23] and noted that, the amount of vitamin E that can enter cellular lipid membranes may be limited by the membrane lipid composition rather than its supply. Probably post administration, vitamin E accumulates slowly, taking weeks to achieve small increments in tissue concentrations.

Kinnula [24] noted that, cigarette smoke causes significant oxidant stress which was further enhanced by recruitment and activation of inflammatory cells to the lung. Some antioxidant enzymes are inducted, but the extent of induction is insufficient to protect the lung/alveolar epithelium against cigarette smoke. Exogenous antioxidants such as vitamins did not seem to protect against cigarette smoke related lung injury. Synthetic compounds with superoxide dismutase and catalase activities have shown promising results in animal models against a variety of oxidant exposures including cigarette smoke in the lung.

Cho HY et al [25] worked on application of Nrf2; nuclear factor E2-related factor 2 in germ-line mutant mice and elucidated protective roles of Nrf2 in various models of human disorders in the liver, lung, kidney, brain, and circulation. Nrf2-ARE(antioxidant response element) binding regulates the expression of more than 200 genes involved in the cellular antioxidant and anti-inflammatory defense such as phase 2 detoxification enzymes

Conclusions

Present study shows significant decrease in antioxidant enzymes SOD, CAT, PON-1 with significant increased levels of MDA as degree of lipid peroxidation and oxidative stress. Moderate changes observed in GPx and GRx levels in asthmatic children as compared to control could be taken as markers of severity and progression of the disease condition.

Further in vitro study and more clinical trials to elucidate a key mechanism by which antioxidant enzyme defences may be enhanced via upregulation of Nrf2, and activation of transcription factor responsible for the expression of antioxidant response element (ARE)-regulated genes are essential.

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