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Biochemical and Antioxidative Response of Asthmatic Patients Receiving Salbutamol

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ABSTARCT

Asthma is a complicated, heterogeneous airway disease. Chronic asthma is often caused due to exercise. Another common class of asthma is allergic asthma which is caused by allergens. Ninety percent asthma cases in children and fifty percent in adults are induced by allergens. Over production of reactive oxygen species in asthmatics lead to the alterations in antioxidants and affect the concentration of electrolytes. Salbutamol (β_2-adrenoreceptor agonists) is the most effective bronchodilators used in the bronchial asthma. The objective of this study was to investigate the electrolytes (Na^+, K^+) disorders, anti-oxidative status malondialdehyde (MDA) levels and their role in hepatic function and lipid profile in male asthmatic patients receiving salbutamol. The study was carried out on sixty two asthmatic patients already receiving salbutamol. The MDA level was significantly elevated (72%) while SOD and GSH concentration were decreased by 92% and 26%, respectively in the asthmatic patients. Serum potassium level was significantly decreased (61%) while sodium level increased (20%). Liver function test like ALT, AST, ALP and total bilirubin increased prominently. Among lipid profile triglycerides and LDL level was raised by 31% and 27%, respectively while HDL showed 32% decrease. Present study suggests that increase in the serum MDA and sodium levels and decrease in potassium GSH and SOD levels triggers the asthma.

Key words: Asthma, β_2-adrenoreceptor, malondialdehyde, anti-oxidants.

INTRODUCTION

Asthma is a complicated, heterogeneous disease which is not easy to define and classify. It is often diagnosed by specific symptoms *i.e.* wheezing, tightness of chest, breath shortness, coughing or inflammation in the airways (Masoli *et al*., 2004; Rabe *et al*., 2004). Asthma is common in the children of different ages, with transient early wheeze which remits before the age of six and late onset of wheeze which starts in the children after three years old and known as persistent wheeze (Martinez *et al*., 1995). For a long time it was considered that asthma is caused by the inflammation produced by eosinophils (Barnes, 1989). In recent studies it was found that non-eosinophilic inflammation also exist which is often caused by neutrophils due to which it is related to asthma (Douwes *et al*., 2002). Remarkable variations present in asthmatic patients that elicit airway narrowing and symptoms of disease, severity of disease and effectiveness of different modes of therapies (Drazen *et al*., 2000; Szefler *et al*., 2002; Rabe *et al*., 2004).

Asthma can be classified in various classes like exercise induced asthma caused due to physical exertion or over exertion, asthma occurring at night also called night time asthma may led to seriousness. Chronic asthma often caused due to exercise. Another class of asthma known as allergic asthma is a common type of asthma caused due to allergens. Ninety percent asthma cases in children and fifty percent in adults are caused by various

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allergens. Different types of allergens are found in the atmosphere (pollen, mites, molds, smoke and sprays) and are present everywhere. In case of allergic asthma, airways are sensitive to the allergen and able to sensitize the allergen. When the allergen enter into the airway, they become inflamed and thick layer of mucus is filled in the airways due to which coughing, wheezing, shortness of breath and chest tightening occurs. Irritants causing asthma also stimulate asthma attack. In case of exercise induced asthma, breathing may occur through the mouth instead of nasal passage (Kattan *et al*., 1978).

In industrialized countries, prevalence of asthma is continuously increasing and in developing and low income countries, consistent data is also available (Starchan *et al*., 1997 and Okudaira, 1998). Another type of asthma known as bronchial asthma that often induced during pregnancy about 1-7% in all pregnancy cases (Alexandar *et al*., 1998; Gordon *et al*., 1970). In two percent cases, asthma is considered the life threatening disorder but this figure is not accurate (Hernandez *et al*., 1980). If asthma is not treated during pregnancy then it may lead to adverse effects on mother and fetus (Turner *et al*., 1980). Epinephrine or non-epinephrine targets on the adrenergic receptors and these receptors are considered as important components in sympathetic nervous system for homeostasis maintenance against disease. Adrenergic receptors belong to the cell surface receptors super-family in which signals are carried out through coupling to guanine-nucleotide binding proteins also called g-proteins. Nine subtypes of adrenergic receptors are present in humans (α_1A, α_1B, α_1Dar, α_2A, α_2B-, α_2Car, β_1, β_2 and β_3AR). Different classical pathways present for coupling of different receptors (coupling of α_1AR occur through stimulation of phospholipase C pathway (Gq), coupling of α_2AR through inhibition of adenylyl cyclase (Gi) and coupling of βAR occur through stimulation of adenylyl cyclase (Gs) (Hoffman *et al*., 2001; Goldstein *et al*., 1998).

β_2-adrenoreceptor agonists (β_2-agonists) are the drugs which are used in the bronchial asthma and are considered the most effective bronchodilators. For ancient times, adrenergic activity containing plants were used in medicine but in modern researches, adrenergic pharmacology started with the isolation of epinephrine from adrenal glands at the end of 19th century (Rau, 1994; Svedmyr *et al*., 1994 and Popa, 1986) and efficient aerosolization devices also introduced (McFadden, 1995) and also found that β_2-agonist used for asthma treatment in three different ways *i.e.*, selection of receptor for reduce side effects produced from activation of non-target receptors, 2nd way is the direct tissue delivery to overcome side effects produced from activation of target receptor in non-target tissues and third way is to increase the duration of action to increase convenience and eliminate night time awakening.

DNA, lipids as well as protein damage is caused by the oxidative stress and in the progression and development of many diseases *i.e.,* asthma and oxidative stress play a key role. In this present research, different biomarkers of oxidative stress were measured in the asthmatic patients *i.e.*, superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH) and catalase (CAT). High production of reactive oxygen species (ROS) is controlled by the mechanism called as anti-oxidant detoxifying mechanism. Two types of mechanisms, one is called as enzymatic (SOD and CAT) and other is called as non-enzymatic (GSH) (Benzie and Strain, 1996 and Cao and Prior, 1998). The objective of this study was to investigate the electrolyte disorders, anti-oxidative status, liver function and lipid profile in in male asthmatic patients receiving salbutamol.

MATERIALS AND METHODS

Blood collection

Blood samples (5ml) were taken from 62 asthmatic patients and 10 normal subjects was processed for the estimation of reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), electrolytes sodium (Na^+) and potassium (K^+) concentration. Lipid (total cholesterol, TCh; triglycerides, TG; Low-density lipoprotein, LDL; and high-density lipoprotein, HDL) and hepatic profile (Alanine aminotransferase, ALT; aspartate aminotransferase, AST; Alkaline phosphatase, ALP and total bilirubin) were estimated by commercially available diagnostic kits.

The activity of SOD was measured by

spectrophotometric method of Kakkar *et al.*, (1984). MDA was measured by the method of Ohkawa *et al.*, (1979). The CAT activity was measured by spectrophotometric method of Aebi, (1984). GSH was measured by the method of Moron *et al*., 1979. The concentration of sodium and potassium was measured by the flame photometer. Independent student t-test was applied for the determination of significant difference between the two groups ($P<0.05$).

RESULTS

The results presented in Table 1 reflect the hepatic function, antioxidative status, lipid as well as electrolyte profile in asthmatic patients receiving salbutamol shows highly significant differences when compared to normal subjects.

Amongst transaminases the ALAT level showed 24% increase as compared from control (31.75±19.73 U/L) value but it was statistically non-significant ($P<0.05$). The ASAT was also elevated (39%) in the asthmatic patients from the control (20.25±5.21U/L) and it was statistically significant ($P<0.05$). The ALP level in the asthmatic patients was remarkably increased (67%) from the control (55.83±6.28U/L). Total bilirubin of the asthmatic patients showed 47% increase from its respective control (1.01±0.07 mg/dL) values (Table 1).

In the present study on lipid profile (TCh, Tg, LDL, HDL) of the asthmatic patients, it was observed that the level of the TCh was raised (21%) from the healthy controls (4.44±0.37mg/dL). The TG level was also increased (31%) in the patients suffered from asthma. The LDL level of the asthmatic patients was raised (27%) from the control which is 2.31±0.15mg/dL. Contrary to LDL, the HD level of the asthmatic patients was decreased (32%) non-significantly from the healthy individuals with 1.73±0.17mg/dL HDL value (Table 1).

Hepatic profiles of asthmatics patients also showed significant changes the activity of ALAT and ASAT were increased by 24% and 31% respectively. The activity of ALP showed highly significant rise of 67% and 47% respectively while bilirubin showed 32% increased. Both electrolytes (Na^+, K^+), the serum potassium showed (61%) significant decrease. Moreover, sodium (177.78± 2.88) concentration was increased 21% from control value (143.15±8.26mEq/L). On the other hand, the levels of antioxidants (SOD and GSH) were decreased (92% and 26%) respectively in asthmatics while MDA level was increased as compared to control (1.36±0.03). Like antioxidants, potassium (1.99±0.03) level was also decreased by 72%.

DISCUSSION

Several lines of evidence suggest that environmental pollutants and oxidants induce oxidative damage in mitochondria of in airway epithelial cells. Exposure of oxidants and allergens induces airway inflammation, resulting in the release of pro-inflammatory mediators, including histamine and leukotrienes (Comhair *et al*., 2005).

Different factors have been reported to play role in the progression of asthma, such as chemicals pollutants, radiations and the genetic makeup by variety of molecular mechanisms. Two important factors are involved in DNA damage which are reactive oxygen species (ROS) and reactive nitrogen species (RNS). Reactive oxygen species and free radicals have long been known to be mutagenic. This damage called oxidative stress or oxidative damage, depends not only on ROS/RNS levels but also on the body's defense mechanisms against them mediated by various cellular antioxidants (Benzie and Strain, 1996; Cao and Prior, 1998).

The findings during this work revealed that the levels of several hepatic and lipid enzymes and metabolites were elevated in asthmatics. Moreover, sodium concentration was increased as compared to control. On the other hand, the level of antioxidants such as SOD and GSH were significantly decreased in asthmatic patients while MDA level was increased (Table 1).

In the recent report on mitochondrial dysfunction and oxidative stress of asthmatic peripheral cells and tissues, it was observed that asthmatic patients have low level of anti-oxidative enzymes. Comhair *et al*. (2005) measured various antioxidants in asthmatic patients. The increase in nitric oxide in the asthmatic patients due to increase in inflammatory cytokines, macrophage inflammatory protein-1 in the epithelial lining fluid,

Table 1: **Biochemical and antioxidative response of asthmatic patients receiving salbutamol.**

Variables	Control (n=20)	Asthmatics (n=62)	% increase/ decrease	(P<0.05)
ALAT (IU/L)	24.00±5.69[a]	31.75±1.97	24.40	0.028
ASAT (IU/L)	20.25±5.21	29.50±3.37	31.35	0.011
ALP (IU/L)	55.83±6.28	169.16±1.35	66.99	0.023
Total bilirubin (mg/dL)	1.01±0.07	1.89±0.02	46.56	0.041
Total cholesterol (mg/dL)	4.44±0.37	5.60±0.41	20.71	0.004
Total triglycerides (mg/dL)	1.24±0.15	1.81±0.11	31.39	0.030
LDL (mg/dL)	2.31±0.15	3.18±0.52	27.35	0.007
HDL (mg/dL)	1.73±0.17	1.18±0.04	-31.79	0.001
MDA (µM/ml)	1.36±0.03	4.78±1.70	71.54	0.000
SOD (ng/mL)	0.73±0.025	0.06±0.05	-91.78	0.016
GSH (mg/dL)	9.77±1.17	7.24±0.94	-25.89	0.005
CAT (µM/mol of protein)	4.27±0.73	2.77±0.83	-35.12	0.033
Na^+ (mEq/L)	143.15±8.26	177.78±2.88	19.56	0.013
K^+ (mEq/L)	5.07±1.02	1.99±0.03	-60.74	0.000

[a] Mean ± SEM; Student's `t' test, P< 0.05 was considered as significant.

eosinophilic infiltrate in bronchoalveolar lavage fluid and biopsy specimens have been reported. It was also observed that extracellular glutathione peroxidase (eGPx) was higher in the airways of adult asthmatic subjects than in those of healthy controls and that the source for the increased eGPx was bronchial epithelial cells (Comhair *et al*., 2005).

Oxygen free radicals, which are generated through several enzymatic and non-enzymatic biological reactions in aerobic organisms, attack a wide variety of macromolecules such as lipid, protein, carbohydrate and DNA. Oxidative stress conditions are characterized by an increase in the concentration of free radicals, and the damage they can cause at different levels of the cellular organization. Either the increase in the rate of free radical generation and or the decrease in antioxidant levels lead to oxidative stress conditions.

In the present report MDA and SOD showed a significant (P<0.05) negative correlation (-0.831*). Inverse correlation also exist between MDA vs GSH, CAT, and potassium (K^+) with r values -0.371*, -0.129* and -0.698* respectively which are also statistically significant (P<0.05). Strong, statistically significant (P<0.01) positive correlation exist between SOD *vs.*, CAT, GSH and potassium (K^+) with r values 0.671**, 0.178** and 0.599** respectively (Table 2).

The findings in this study provide an association of asthma with SOD, GSH and serum potassium. The asthmatic patients had found a significantly lower serum level of SOD, GSH and potassium levels as compared to the healthy subjects. The level of SOD in the asthmatic patients was significantly lower as compared to the healthy individuals. The extent of lipid peroxidation in the asthmatic patients was remarkably increased.

Table 2: **Spearman's correlation (r) of different variables in asthematic patients receiving salbutamol**

Parameters	r value
MDA Vs SOD	-0.831*
MDA Vs CAT	-0.129*
MDA Vs GSH	-0.371[ns]
SOD Vs CAT	0.671**
SOD Vs GSH	0.178[ns]
MDA Vs K^+	-0.698*
SOD Vs K^+	0.599*

Significance has been shown as *p<0.05; **p<0.01

The present study showed a relationship between oxidative stress, electrolyte balance and asthma. Biochemical study of the asthmatics showed that oxidative stress and electrolyte balance play a key role in the progression of asthma. The asthmatic patients had remarkably high lipid peroxidation due to which the level of MDA was increased remarkably while the level of SOD, CAT and GSH was decreased. Moreover, hepatic and lipid profile were also statistically significant between the studied groups. It can be suggested from this study that increased MDA and sodium levels and decreased potassium, SOD and GSH levels of the asthmatic patients may contribute to the progression of asthma.

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