

## **A Comparative Study of Metabolic and Antioxidative Response of Salbutamol Therapy in Asthmatic and Non Asthmatic Athletes in Punjab**

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### **ABSTRACT**

**Place of study:** Punjab Institute of Cardiology, OPD and inpatient departments.

**Methods:** A total of 93 patients were included in this study: Out of which 54 patients diagnosed with bronchial asthma and 19 patients diagnosed as non asthmatic athletes receiving salbutamol. None of the patients were taking any antioxidant supplements and did not have any symptoms of lower or upper respiratory tract infection. Those patients who had pulmonary or systemic disease or an acute exacerbation of asthma within the past 4 weeks were excluded from the study. No drug was allowed on the day of testing. Twenty age matched control subjects were selected without respiratory symptoms and normal lung functions. All recruited subjects were nonsmokers and majority of the patients included in the present study were reported in emergency with attack of tachycardia.

**Conclusion:** Salbutamol-induced hypokalaemia becomes the ultimate cause of tachyarrhythmias which result in higher levels of O<sub>2</sub> & stimulate the membrane-bound  $\beta$ 2-adrenoceptor linked Na<sup>+</sup>/K<sup>+</sup> ATPase causing potassium influx into cells.

**Keywords:** Salbutamol,  $\beta$ 2-adrenergic, Antioxidative Status

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### **INTRODUCTION**

Salbutamol or albuterol ( $\beta$ 2-adrenergic receptor agonist) is used for the relief of bronchospasm in conditions such as asthma and chronic obstructive pulmonary disease (COPD). It is available as Ventolin among various brand names. Salbutamol sulfate is usually given by the inhaled route for direct effect on bronchial smooth muscle. This is usually achieved through a metered dose inhaler (MDI), nebulizer or other proprietary delivery devices<sup>1</sup>. High doses may cause hypokalaemia<sup>2</sup> which is of concern in patients with renal failure and those on certain diuretics and xanthine derivatives. Salbutamol is taken by some as an alternative to clenbuterol for purposes of fat burning, and/or as a performance enhancer. Studies have shown very similar dose-response relationships to  $\beta$ 2-agonists in normal and asthmatic subjects<sup>3,4</sup>. Salbutamol could also be more sensitive to endogenous adrenaline, which may dilate and stabilize airways<sup>5</sup>.

### **PATIENTS AND METHODS**

This study was conducted in the Punjab Institute of cardiology, on patients attending OPD and in patient departments (IPD). Total 93 patients were included in this study: Out of which, 54 patients reported with

bronchial asthma and 19 patients reported non asthmatic athletes receiving salbutamol. Twenty age matched control subjects were selected without respiratory symptoms and normal lung functions.

Plasma level of MDA was measured by measuring thiobarbituric acid reactive substances (TBARS) according to the method described by Ohkawa et al (1979)<sup>6</sup>. For assay, 0.5 ml of plasma sample was mixed with equal volume of 0.67% TBA and 30% of TCA in tubes. The mixture was heated in a boiling water bath for 20 minutes. The tubes were centrifuged at 4320 g for 15 minutes. The absorbance of the pink supernatant was taken at 530nm on the UV-visible spectrophotometer. Results were expressed in nmol of MDA/ml of plasma.

The quantitation of catalase activity in erythrocyte hemolysates was based on the oxidation of H<sub>2</sub>O<sub>2</sub><sup>7</sup>. The activity of catalase was measured by taking 1.95 ml of potassium phosphate buffer (50mM, pH 7.0), 50 $\mu$ l of the sample to which 1ml of H<sub>2</sub>O<sub>2</sub> (30mM) was pipetted in dark conditions. The solution was immediately read at 240nm for 3 minutes by UV-visible spectrophotometer. The assay was carried out according to the method of Moron *et al.*, (1979)<sup>8</sup>, in which the reaction was initiated by addition of DTNB and the rate of reduction was measured at 412 nm on UV-visible spectrophotometer.

### **RESULTS**

The results regarding hemoglobin and RBC count (Table 1) demonstrating statistically highly significant difference of hemoglobin and decreasing (RBC)

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pattern between the asthmatic vs non asthmatic athletes receiving salbutamol subjects (P=.05, .012 and .007 respectively). The lowest value of hemoglobin was recorded in asthmatic patients and highest in control. A slightly decreasing trend was also observed in non-asthmatics. The highest value (5.97) of RBC count ( $\times 10^6$ ) was found in control followed by non asthmatics athletes (4.02  $\times 10^6$ ) and asthmatic patients (4.75  $\times 10^6$ ) receiving salbutamol.

The data regarding blood urea and creatinine reflecting highly significant differences but inconsistent increasing and decreasing pattern between asthmatic vs non asthmatics athletes receiving salbutamol subjects (P=.05, .014 and .023 respectively). The lowest value of blood creatinine was recorded in control and highest in asthmatics followed by a slightly decreasing trend was observed in non-asthmatic athletes. Increasing trend of blood urea was recorded in asthmatic and non-asthmatic athletes respectively with a normal value in control.

From the results presented in Table 1, shows that all the circulating enzymatic biochemical markers e.g. ALT, AST, alkaline phosphatase (ALP) and total bilirubin exhibited highly significant (P=.05, .028, .011, .023 and .041 respectively) difference not only between the study groups but also within the same group. The highest values of ALT, AST and TB were recorded in asthmatic patients and except ALP in non asthmatic athletes receiving salbutamol. Lipid profile

of asthmatics vs non asthmatic athletes differ significantly (P=.05, .044, .030, .007 and .001 respectively). An increasing trend of HDL, TG and LDL was observed both in asthmatic and non asthmatic athletes as compared to controls but a decreasing trend of Cholesterol was recorded in both the study groups

Data regarding stress biochemical markers MDA (Malondialdehyde), SOD (Superoxide dismutase), GSH (Reduced glutathione) and CAT (Catalase) shows highly significant pattern among the study groups. The consistent increasing trend in MDA levels were recorded in asthmatic and non-asthmatic athletes as compared to control. The consistent decreasing trends in case of reduced glutathione from study groups were recorded. Catalase levels showed the decreasing trend in different study groups. The lowest value (0.06ng/ml) of SOD was recorded in asthmatic patients and in non asthmatic athletes (0.12ng/ml). Data regarding blood glucose level of asthmatic vs non asthmatic athletes receiving salbutamol showed that fasting blood glucose levels of non asthmatic athletes have increasing trend as compared to control while asthmatic patients also have the lowest value of blood glucose levels. Data regarding electrolytes in asthmatic vs non asthmatic athletes receiving salbutamol shows highly significant difference between and within the study groups.

Table 1: Hematological and Biochemical Profile In Asthmatic and Non-Asthmatic Athletes Taking Salbutamol

Variables	Control (n=20)	Asthmatic (n=54)	Non-Asthmatic Athlete(n=19)	(P<0.05)
Hb	14.9±0.99	11.3±1.4	12.8±1.2	.012*
RBC	5.97±0.5	4.8±0.6	4.02±0.4	.007*
ALT	24.0±5.7	31.8±19.7	27.04±21.2	.028*
AST	20.3±5.2	29.5±9.3	22.2±31.1	.011*
ALP	55.8±6.3	169.2±13.5	189.3±23.2	.023*
Total Bilirubin	1.0±0.1	1.9±0.02	1.8±0.2	.041*
Cholesterol	4.4±0.4	5.6±0.4	5.2±0.5	.004*
Triglycerides	1.2±0.2	1.8±0.1	1.9±0.3	.030*
LDL	2.3±0.2	3.2±0.5	4.9±0.4	.007*
HDL	1.7±0.2	1.2±0.04	1.2±0.2	.001*
Urea	20.7±1.5	22.5±2.2	24.4±2.14	.014*
Creatinine	0.96±0.2	1.1±0.1	1.01±0.23	.023*
Malondialdehyde (MDA)	1.4±0.03	4.8±1.7	5.3±1.7	.000*
Superoxide dismutase (SOD)	0.7±0.03	0.1±0.1	0.12±0.01	.000*
Glutathione (GSH)	9.8±1.2	7.24±0.9	5.04±0.9	.005*
Catalase (CAT)	4.3±0.7	2.8±0.8	3.7±1.4	.033*
Glucose	105.2±5.6	97.2±4.3	122.3±7.3	.001*
Ca <sup>++</sup>	12.03±3.3	13.3±3.7	12.9±1.9	.036*
Na <sup>+</sup>	143.2±8.3	177.8±2.9	144.9±3.9	.013*
K <sup>+</sup>	5.1±1.02	1.99±0.03	2.56±1.8	.000*
Mg <sup>++</sup>	3.5±0.2	1.9±0.5	1.8±0.9	.058
Cl <sup>-</sup>	107.3±10.4	117.9±8.9	115.9±4.9	.076

Hb=g/dl, RBCs=  $\times 10^6$  /mm<sup>3</sup>, ALT=IU/L, AST=IU/L, ALP=IU/L, Total Bilirubin=mg/dl, Total cholesterol=mg/dl, Triglycerides=mg/dl, LDL=mg/dl, HDL=mg/dl, Urea=mg/dl, Creatinine= mg/dl, MDA=nM/ml, SOD=ng/ml, GSH=mg/dl, CAT= $\mu$ M/mol of protein, Glucose=70-100 mg/dL, Na<sup>+</sup>=132-142 mEq/L, K<sup>+</sup>=4.0 - 4.7 mEq/L, Ca<sup>++</sup>=9-11 mEq/L (Ionized: 3.9-4.5 mg/dL), Cl<sup>-</sup>=95-105 mEq/L, Mg<sup>++</sup>=1.8-3 mg/dL

## DISCUSSION

There were marked differences in biochemical parameters and other demographic variables among patients with different severities of asthma except age, sex, and SOD activity. Pearson's correlation coefficients of oxidative variables showed remarkable negative correlation between plasma TBARS and blood glutathione in patients with asthma as well as in controls. A remarkable positive correlation was found between total protein sulfhydryls, erythrocyte GPx, and total antioxidant status in asthmatic patients as well as in controls. Conversely, SOD ( $r = -0.07$ ,  $P > 0.05$ ) exhibited a slight negative correlation, whereas catalase showed an insignificant positive correlation in asthmatic patients ( $r = 0.01$ ,  $P > 0.05$ ). Several studies have revealed that oxidant-antioxidant balance is an essential factor for the normal physiological function of the lungs.

An increased oxidant and/or decreased antioxidant may reverse the physiologic oxidant-antioxidant balance in favor of oxidants. Thus, oxidative stress plays an important role in the pathogenesis of asthma and oxidants caused lipid peroxidation by oxidizing fatty acids and polyunsaturated lipids of cell membranes. The oxidant-antioxidant status was investigated in blood (red blood cell and plasma) because it is an easily available source and also considered as an important pool of antioxidant defenses in the body rather than BAL fluid which is obtained through an invasive bronchoscope technique. Furthermore, asthma is an inflammatory disease in which cells are recruited from the peripheral blood. In this work, it has been observed that the asthmatic patients have increased oxidative stress. It was shown by remarkable increase in lipid peroxidation product (MDA) and protein carbonyls as well as decrease in protein sulfhydryls in plasma.

The distinguished changes were noticed in catalase activity and total antioxidant power of plasma of asthmatic patients. Rahman et al (2006)<sup>9</sup> reported that the plasma MDA level was higher in asthmatic patients than controls as well as in patients with asthma exacerbation as compared to stable asthma. Similarly, another study entails that MDA level in 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. Several studies have demonstrated alterations in different endogenous antioxidants in patients with asthma. The alterations in antioxidant defenses may involve either an increase or a decrease depending on the changes occurring due to a defense response. SOD is an intracellular antioxidant enzyme, helping in removing superoxide

anion. In some studies, it has been reported that SOD activity in airway epithelium and erythrocytes of asthmatic patients was lower than healthy controls.

It was also observed a dramatic enhancement in catalase activity among asthmatic patients (intermittent to severe). Al-Abdulla et al (2010)<sup>10</sup> 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. The association between asthma severity and anthropometric measurement demonstrates that age and gender was unrelated to disease severity. The results are also of interest in the context of increased oxidative burden remarkably associated to the pulmonary airways obstruction. Menon et al (2004)<sup>11</sup> found a remarkable negative correlation between plasma TBARS and red cell GSH, but they did not find any significant correlation with GPx and total antioxidant status.

## CONCLUSION

This study supports the hypothesis that salbutamol induces hypokalaemia which becomes the ultimate cause of tachyarrhythmias. Positive correlation between superoxide dismutase and potassium [SOD Vs  $K^+$   $r = .599$  ( $P = .037$ )] also support the hypothesis that high levels of  $O_2$  stimulates the membrane-bound  $\beta_2$ -adrenoceptor linked  $Na^+/K^+$  ATPase pump thus promoting potassium influx into cells.

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