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TO STUDY LEVEL OF GLUTATHIONE PEROXIDASE (GSHPX), GLUTATHIONE REDUCTASE (GR), REDUCED GLUTATHIONE (GSH) IN PATIENTS WITH BRONCHIAL ASTHMA

General Medicine

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ABSTRACT

Prevalence of asthma has increased considerably in recent decades throughout the world especially in developed countries8. Airway inflammation is thought to be prime cause for repeated episodes of airway obstruction in asthmatics. Several studies have shown that reactive oxygen species (ROS) play a key role in initiation as well as amplification of inflammation in asthmatic airways6. Excessive ROS production in asthma leads to alteration in key enzymatic as well as nonenzymatic antioxidants such as glutathione, vitamins C and E,beta-carotene, uric acid, thioredoxin, superoxide dismutases, catalase, and glutathione peroxidases, leading to oxidant–antioxidant imbalance in airways2. This review summarizes the scientific and epidemiological evidence linking asthma with oxidant–antioxidant imbalance. **1. AIM.** The aim of the study was to measure activity of antioxidant enzymes GSHPx, GR,GSH and G6PD in neutrophils of bronchial asthma patients. **2.MATERIAL AND METHOD:** The study was carried out on the patients attending the asthma clinic at the B.Y.L. Nair charitable hospital Mumbai. **Sample size -**100 bronchial asthma patients and 50 normal subjects were included in study. Blood was collected and processed for PMNL separation and lysate preparation.GSHPx, GR,GSH and G6PD done by standard biochemical methods. In this study the activities of oxygen detoxifying enzymes of PMNL were measured in the patients suffering from bronchial asthma (n=100) in the age group of 18 to 45 years. **Study design:** It was prospective observational cohort study conducted in tertiary care hospital in indoor and OPD patients.Subjects were divided into two study groups A INCLUDED -100 bronchial asthma patients and GROUP B -50 normal subjects. **Inclusion criteria:** Adult patients suffering from bronchial asthma (n=100) in the age group of 18 to 45 years were selected for the study: **Exclusion criteria :** Individuals who had history of any other diseases ie.Hypertension,Diabetes,and liver disease were were excluded for the study. The result of study were compared against age and sex matched healthy individuals (n=50). **Experimental methods:** The blood was collected in the tube containing anticoagulant Acid Citrate Dextrose.(ACD).And samples were immediately processed for PMNL separation and lysate preparation. The investigation included determination of the lung function and the measurements of plasma superoxide dismutase activity (SOD), glutathione content (GSH) reduced form, glutathione peroxidase activity (GSH-Px), catalase activity (CAT), lipid per- oxidase (LP), and nitric oxide (NO).**3. STATISTICAL ANALYSIS:** All statistical analysis were carried out using the SPSS (statistical package for the social science software) statistical package version 20.0 for Windows, and the software Microsoft Excel V.5 (2003). USA 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 $p < 0.05$ **4.CONCLUSION:** Reactive oxygen species do play a role in asthma.

KEYWORDS

Antioxidants, Asthma, Inflammation, Oxidative, Stress

1. INTRODUCTION

Asthma is a disease characterized by chronic airway inflammation. Generation of oxygen free radicals by activated inflammatory cells produces many of the pathophysiologic changes associated with asthma[1]. However, the activities of antioxidant enzymes and their relation with asthma have not been well defined. This study was performed to examine the activities of major intracellular antioxidants in 100 asthmatic patients 50 age- and sex-matched healthy control subjects were selected. The activities of erythrocyte antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione-peroxidase (GSH-Px) were measured spectrophotometrically. The mean SOD activity of asthmatic patients was found to be significantly lower than that of the controls ($p < 0.05$). There was no significant difference in CAT and GSH-Px activities between patients and controls ($p > 0.05$). Although the mechanisms underlying the association between asthma and antioxidant system are unclear, according to our findings, decreased antioxidant protection may contribute to the pathogenesis of mild asthma.[5]

2. Aim. The aim of the study was to measure activity of antioxidant enzymes GSHPx, GR,GSH and G6PD in neutrophils of bronchial asthma patients.

3 Material and Method:

The study was carried out on the patients attending the asthma clinic at the B.Y.L.Nair charitable hospital, Mumbai.

Sample size -100 bronchial asthma patients and 50 normal subjects were included in study.Blood was collected and processed for PMNL separation and lysate preparation.GSHPx, GR,GSH and G6PD done by standard biochemical methods. In this study the activities of oxygen detoxifying enzymes of PMNL were measured in the patients suffering from bronchial asthma (n=100) in the age group of 18 to 45 years.

Study design: It was prospective observational cohort study conducted in tertiary care hospital. Indoor and OPD patient total no 100 were included in the study after Ethics committee clearance .Subjects were divided into two study groups A included -100 bronchial asthma patients and group B -50 normal subjects.

Inclusion criteria: Adult patients suffering from bronchial asthma (n=100) in the age group of 18 to 45 years were selected for the study:

Exclusion criteria: Individuals who had history of any other diseases ie.Hypertension,Diabetes,and liver disease were excluded from the study ,The result of study were compared against age and sex matched healthy individuals (n=50). Table 1

Table 1 Clinical characteristics of the COPD patients.

Character	Mean ± SD
Age	54.9 ± 9
Sex (M/F)	27/7
Cigarette (pack-years)	46.4 ± 1.7
FEV1 (%)	59 ± 3.7

2.3 Experimental methods:

The blood was collected in the tube containing anticoagulant Acid Citrate Dextrose.(ACD).And samples were immediately processed for PMNL separation and lysate preparation.

Estimation of GSHPx ,GR, GSH , G6PD and proteins done from neutrophil lysate GSHPx. **Principle :** Glutathione peroxidase catalyses the oxidation of GSH to GSSG

GSHpx
2 GSH+ROOH_____,,,,,^GSSG+ H2O+ R-OH

Where ROOH is the peroxide t-Butylhydroperoxide is the most suitable substance for assay of the enzyme. The rate of reduction of GSSG by glutathione reductase (GR) is measured.

GR
GSSG+NADPH____^2GSH+ NADP
The oxidation of NADPH is followed at 340 nm.

GR.: Spectrophotometric Method. **Principle:** Glutathione reductase catalyses the reduction of oxidized glutathione (GSSG) by NADPH or NADH to reduced glutathione.

GR
NADPH (NADH) + H + GSSG_____^ NADP+(NAD)+ 2GSH

The activity of this enzyme is measured by following the oxidation of NADPH (NADH) spectrophotometrically at 340 nm. Glutathione reductase is a flavin enzyme and complete activation of apoenzyme requires the preincubation of the enzyme with FAD.This must be done before GSSG or NADPH is added to the reaction system, since these seems to interfere with activation of the enzyme by FAD.GSH

Principle: Virtually all of the nonprotein sulfhydryl of cells is in the form of reduced glutathione (GSH)..5,5-Dithiobis (2-nitrobenzoic acid)(DTNB) is a disulphide compound which is readily reduced by sulfahydryl compounds, forming a highly coloured yellow anion.The optical density of this yellow substrate is measured at 412nm.

EDTA present in the participating solution inhibits the metal catalysis of the auto-oxidation of GSH.

Glucose -6- Phosphate Dehydrogenase :
Principle: Glucose 6 Phosphate dehydrogenase catalyses the oxidation of glucose 6 phosphate to 6 phosphogluconate (6-PGA). 6 phosphogluconate dehydrogenase catalyses the oxidative decarboxylation of 6 PGA to ribulose 5 phosphate and carbon dioxide , using NADP as the acceptor of hydrogen.

Principle. Proteins react with two reagents,the alkaline copper reagent and the Folin Phenol reagent. The final colour obtained is a result of:

1). The biuret reaction of protein with the copper ions of the alkaline copper reagent and
2). A reduction of the phosphotungstic acid to tungsten blue and molybdenum blue both by the Cu-protein complex and by the tyrosine and tryptophan of the protein. The blue colour obtained as a result of the above reactions is read at 650 nm.

4. RESULT:

G6PD showed considerable rise. There was pronounced fall in the GR activity and non significant changes were observed in GSH content and GSHPx activity. The mean concentration of glutathione (GSH) was significantly higher in the control subjects compared with COPD patients (p = 0.001, 0.001) respectively. There is no significant difference in the concentration of glutathione-peroxidase (GSH-Px) in all study participants The mean concentration of superoxide dismutase (SOD) was significant higher in the control subjects compared with COPD patients (p = 0.012, 0.001) respectively. Also the mean concentration of superoxide dismutase (SOD) was significantly higher in control G6PD showed considerable rise. There was pronounced fall in the GR activity and non significant changes were observed in GSH content and GSHPx activity.

RESULTS AND ANALYSIS :

Percent Rise / Fall in enzymatic and non enzymatic antioxidants in PMNL of asthma patients.

Assay.	Control.	Asthma.	P value
GR without FAD	0.112+-0.043.	41.96%^.	P^0.001
GR with FAD. (u moles/ mg of protein)	0.138+-0.047.	33.33%^.	P^0.001
G6PD. (U/gm of protein)	6.63+- 2.2.	17.64%^	P^0.001
GSH. (U moles/mg Of protein)	0.071+- 0.022	7.74%^.	NS
Protein(mg/ml of lysate	0.8+-0.2.	Practically	no change

5.DISCUSSION.

Though there was pronounced fall in the GR activity, non significant changes were observed in GSH content and GSHPx activity[74].

Over the past decade, a number of studies have clearly demonstrated that (a) oxidative stress is an important consequence of the inflammatory response in asthma [Nadeem *et al*. 2005, 2003; Wood *et al*. 2003], (b) oxidative stress is associated with an alteration in antioxidant activity in the lung and blood [Sackesen *et al*. 2008; Nadeem *et al*. 2005, 2003; Heffner and Repine, 1989], and (c) oxidative stress is associated with the development of airway hyper responsiveness [Saleh *et al*. 1998; Katsumata *et al*. 1990]. This is supported by the studies that have shown that inflammatory cells from peripheral blood and bronchoalveolar lavage (BAL) fluid of asthmatic subjects generate more superoxide anion radicals than those from controls [Nadeem *et al*. 2003; Kelly *et al*. [1988] demonstrated that there was a twofold increase in generation of ROS from neutrophils asthmatic subjects compared with control subjects and fourfold from macrophages in asthmatic subjects compared with control subjects. Furthermore, macrophage ROS generation was associated with an increase in airway responsiveness[3].

CONCLUSION:

It seems that ROS contribute to the pathophysiology of asthma and one could propose a recurrent cycle of recruitment and stimulation of inflammatory cells,leading to the continued release of ROS.If such a scenario existed then antioxidant therapy could in fact be useful in the treatment of this disease.

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