BIOCHEMICAL STUDY ON ENZYMATIC ANTIOXIDANT IN ASTHMATICS

A. G. Rajalakshmi*

Molecular Diagnosis and Drug Discovery Laboratory, Department of Biotechnology, School of Life Sciences, Karpagam University, Coimbatore, Tamilnadu, India.

ABSTRACT

Oxidative stress contributes to the pathophysiology of respiratory disorders and other chronic diseases associated with asthma. Increased levels of oxidants are considered a marker of the inflammatory process. The objective of the present study was to investigate the levels of antioxidants status in asthmatic patients in comparison with healthy volunteers. Lipid peroxidation assay, Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Catalase (CAT), was determined in 95 asthmatic patients based on severity. The results of the present study suggest that downward and elevated level of antioxidants are associated with the severity of asthma. The results revealed the existence of an increased oxidative stress and reactive oxygen species in asthmatics, with altered antioxidant capacities.

KEY WORDS: MDA, SOD, GPx, CAT, Asthma.

INTRODUCTION

The lung has a large surface area exposed to greater oxygen free radicals. The mechanism by which oxygen radicals cause asthma pathology is by oxidation or nitration of proteins, lipids, or DNA to cause Dysfunction of molecules. Increased susceptibility to the effects of reactive oxygen species (ROS) generated by inflammatory cells recruited into the Lungs\(^1\), which leads to oxidative stress an important consequence of the inflammatory response in asthma\(^2,3\), and it is associated with an variation in antioxidant activity in the lung and blood.\(^4\) The first reactive oxygen species produced in the reduction pathway of oxygen to water is the superoxide anion, which participates in the generation of other toxic metabolites, most importantly hydrogen peroxide (H\(_2\)O\(_2\)) and hydroxyl radical. The important H\(_2\)O\(_2\)
scavenging enzymes include catalase and the glutathione peroxidases, the latter ones being closely associated with the maintenance of reduced glutathione.

The current study was aimed to evaluate the hazardous effects and assessment of the oxidative stress by antioxidant parameters caused by lipid peroxidation, Superoxide dismutase, catalyses dismutation of superoxide anion into hydrogen peroxide (H₂O₂). Both catalase and glutathione peroxidase (GPx) activity detoxifies hydrogen peroxide. Additionally, the present work aimed to evaluate the levels of the antioxidant enzymes in asthmatics.

**MATERIALS AND METHODS**

**Study subjects**

Ninety five asthmatic subjects, aged 18-45yrs, were recruited using informed consent. The study was evaluated for smoking history, family history of asthma and allergies (taking into account the first-degree relatives), accompanying disease, and use of medications.

**Samples**

Blood samples (5 ml) were obtained by venipuncture from asthma subjects based on severity for the study. The blood was collected in a tube containing sodium citrate. Serum and plasma were obtained respectively by centrifugation at 3500 rpm and was stored for further analysis.

**Lipid peroxidation assay**

The TBARS level were estimated, by adding, 0.5 ml of plasma, 0.5 ml of normal saline, 1 ml of 20% trichloroacetic acid (TCA) and 0.25 ml of TBA reagent (200 mg of thiobarbituric acid in 30 ml distilled water and 30 ml of acetic acid) were added incubated for one hour. To each of the test tubes, 3 ml of n-butanol was added centrifuged at 3000 rpm for 10 minutes. The separated butanol layer was collected and read at 535 nm.[5]

**Assay of Superoxide Dismutase (SOD)**

Estimation of plasma SOD, by adding of double distilled water, 0.5µl of plasma, 1.2 ml of sodium pyrophosphate buffer (pH 8.3), 0.1 ml of phenazine methosulphate (PMS) and 0.3 ml of nitroblue tetrazolium (NBT) were added and mixed. 0.2 ml of NADH solution was added to it to initiate the reaction. After incubation the reaction was terminated by adding 1 ml of glacial acetic acid followed by 4 ml of n -butanol was added and the mixture was centrifuged and the absorbance recorded at 560 nm.[6]
Assay of Glutathione Peroxidase (GPx)

GPx activity was measured 0.5 mL serum sample, an equal volume of double strength Drabkin’s reagent was added and mixed. This mixture (50 μl) was combined with 100 μl of 8 mM NADPH, 100 ml of 150 mM glutathione (reduced form), 20 ml of glutathione reductase (30 U/ml), 20 μl of 0.12 M sodium azide solution, and 2.65 ml of 50 mM potassium phosphate buffer (pH 7.0, 5 mM EDTA) and the tubes incubated and initiated H₂O₂ solution, measured at 340 nm.[7]

Estimation of Catalase (CAT)

Catalase measurement was done by 2.25 ml of potassium phosphate buffer (65 mM, pH 7.8) was added to 0.1 ml of serum and incubated at 25°C for 30 min. 0.4 ml of 2 M H₂O₂ was added to initiate the reaction. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3). The change in absorbance was measured at 240 nm by spectrophotometer.[8]

Statistical analysis

For the descriptive analysis, continuous variables were described by the mean (SD) and categorical variables by the number and percentage of patients in each response category. Results were calculated using Excel (Microsoft Office, Version 2007).

RESULTS

Table I shows the comparison of the three studied groups. The mean levels of MDA, SOD, GPx, catalase in asthmatic patients were analyzed based on the severity. The level of MDA and SOD was significantly raised with increasing in severity of asthma attack. While the level of GPx, catalase were significantly decreased with increasing severity of asthma attack. In aggregate, significantly lower free radical scavenger activity was found in severe asthmatics compared to mild and moderate asthmatics (P<0.05).

Table I: Level of enzymatic antioxidant in asthmatics based on severity.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Asthma patients (N=95)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mild</td>
</tr>
<tr>
<td>Lipid peroxidation assay (µmol/l)</td>
<td>19.3±0.21</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>19.2±1.4</td>
</tr>
<tr>
<td>GPx (U/ml)</td>
<td>13.6±0.6</td>
</tr>
<tr>
<td>Catalase (U/ml)</td>
<td>18.3±1.4</td>
</tr>
</tbody>
</table>
SOD- Superoxide Dismutase, GPx- Glutathione Peroxidase, GSH-Reduced glutathione. Data are expressed in Mean ± SD and t test analysis for comparison of group; *p< 0.05.

DISCUSSION
The lung has high exposure to atmospheric oxygen, i.e. susceptible to oxidative injury and is equipped with strong antioxidants defenses to counteract the oxidant insult. An imbalance between the oxidative forces and the antioxidant defenses system favoring an oxidative injury has been implicated in asthma.\[^9,10\] Increased level of reactive oxygen species such as superoxide (O\(_2^\cdot\)), H\(_2\)O\(_2\) and OH\(^-\) radical inactivate anti-proteases, induce apoptosis and lead to an increased airway reactivity and secretions, production of chemo attractant molecules and increased vascular permeability, which collectively augment the existing inflammation that is the property of asthma.\[^11,12\] The antioxidant pathways that form the major line of defense against the oxidative insult within the lung can be divided into enzymatic and non-enzymatic systems.\[^4,10\] Increasing evidence that ROS generation plays a major pathophysiological role in asthma. Enhancing balance in the intracellular content of antioxidant enzymes like superoxide dismutase, glutathione peroxidase, MDA and catalase can provide means of limiting biological damage caused by oxygen free radicals.

Malondialdehyde, a marker for the oxidant stress was significantly higher in asthma patients when compared with the controls. This observation showed that asthma patients have an increase oxidative stress which is shown remarkably by an increase in lipid peroxidation product (MDA). Kirkham and Rahman (2006) reported that the plasma MDA level was higher in asthma patients than in controls. Similarly, another study entitles that MDA level was higher in mild to severe asthma patients.\[^13\] SOD is a first-line antioxidant essential to aerobic life; loss of enzyme-specific activity undoubtedly potentiates extracellular matrix damage and tissue injury through increased formation of reactive oxygen and nitrogen species due to airway epithelial cells.\[^14,15\] In the present study increased superoxide anion generation due to the over expression of SOD might be an adaptive response and results in increased dismutation of superoxide to H\(_2\)O\(_2\). Similar results could be easily observed that upper limits of SOD activity in asthmatics were recorded within the limit level of controls which ascertained the significant elevation of SOD in asthma patients. The elevation recorded in the present study is in good agreement with previous studies that recorded higher plasma SOD activity in asthmatic patients compared to controls.\[^16-18\] High SOD activity together with low GPx activity in asthmatics confirms the contribution of oxidative stress in the
etiology of asthma as renowned by Liao et al., 2004.[16] Though catalase is reliable for detoxification of H$_2$O$_2$, that formed by SOD and other processes is scavenged by catalase. In the present study a significant decrease in catalase activity was observed in asthma patients.

REFERENCES