

Study of the Serum Superoxide Dismutase Levels in Smoking and Non-Smoking Patients with COPD

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Research Article

Abstract: Setting- The present study was carried out in the department of Physiology in collaboration with department of Biochemistry and Medicine, Govt. Medical College and Hospital, Aurangabad, Maharashtra, India. **Objectives-** To compare the serum superoxide dismutase (SOD) levels among healthy smokers, smoking patients with COPD and non-smoking patients with COPD and to correlate serum SOD levels with the severity of obstruction in COPD patients. **Design-** A total of 120 subjects in the age group of 40-70 years from medicine department of the hospital were included and they were divided into four groups.

Group: 1 - Healthy non-smoking individuals as control.

Group: 2 - Healthy smokers.

Group: 3 - Non-smoking patients with COPD.

Group: 4 - Smoking patients with COPD.

Pulmonary function tests (PFTs) were used for COPD diagnosis as per GOLD criteria and Serum SOD levels were measured by the method devised by Marklund S, Marklund G modified by Nandi and Chatterjee. The analysis of the study was done by using ANOVA, Pearson's correlation coefficient and Unpaired t test. **Results-** The serum SOD levels were significantly decreased in healthy smokers (2.50 ± 0.45 U/ml), non-smoking patients with COPD (2.36 ± 0.49 U/ml) & smoking patients with COPD (2.23 ± 0.43 U/ml) when compared to controls (3.01 ± 0.23 U/ml). Also, serum SOD levels were positively correlated with FEV₁% predicted in smoking and non-smoking patients with COPD. **Conclusion-** These results provide enough evidence of increased oxidative stress and a compromised antioxidant defence system in smokers and in patients with COPD. This study also revealed that the serum SOD levels can act as an indicator of severity of COPD, which may help in prognosis and follow-up of the treatment of patients with COPD.

Keywords: COPD, oxidative stress, serum SOD, FEV₁, GOLD criteria.

Introduction

Chronic obstructive pulmonary disease (COPD), the fourth leading cause of death in the world, represents an important public health challenge that is both preventable and treatable. It is a major cause of chronic morbidity and mortality throughout the world; many people suffer from this disease for years and die prematurely from it or its complications. Globally, the COPD burden is projected to increase in coming years

due to continued exposure to risk factors and aging of the population. COPD has been defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD), as a preventable and treatable disease characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. [1] COPD is rare before the age of 40, but after that age, symptoms of hyper secretion occur with increasing frequency. Persistent airflow obstruction becomes more common at around age of 60. The prevalence of COPD then increases progressively until 60-70 year of age when, in large part due to mortality, it becomes more stable. [2] The chronic airflow limitation characteristic of COPD is caused by a mixture of small airway disease (obstructive bronchiolitis) and parenchymal destruction (emphysema), the relative contributions of which vary from person to person. Airflow limitation is best measured by spirometry, as this is the most widely available and reproducible test of lung function. [1] Oxidative stress due to smoking is said to be the main etiological factor causing COPD in a dose-response relationship to the intensity of cigarette smoking. Smoking has been proven to alter several antioxidant enzymes with potential effects on prevention or progression of the disease. Although the casual relationship between smoking and development of COPD has been absolutely proven, only about 15-20% of the smokers develop COPD, which suggests a role of other stress inducing environmental or genetic factors contributing to the impact of smoking on the development of airflow obstruction. [3, 4, 5, 6] Oxidative stress is due to free radicals that are reactive oxygen and nitrogen species (ROS / RNS) which may react with various molecules and result in either direct damage or produce potentially harmful products and thus play a key role in the pathogenesis of COPD.

However, there are also protective mechanisms to attenuate the deleterious effects of these oxidants. These are the Antioxidants. One of the crucial of them is superoxide dismutase (SOD), which convert superoxide anions into hydrogen peroxide. SODs are the only enzyme family with activity against superoxide radicals. [7] In this study, serum levels of SOD were estimated and compared among the control, healthy smokers, smoking patients with COPD and non-smoking patients with COPD and an attempt was made to find out relationship of smoking with the state of oxidative stress.

Material and Method

The present study is an observational, descriptive study carried out in the departments of Physiology, Biochemistry & Medicine at Govt. Medical College and Hospital, Aurangabad, Maharashtra. The study protocol was approved by the Institutional Ethics Committee of the institute. Before enrollment in the study, informed written consent was obtained from each subject. All the subjects were in the age group of 40 to 70 years, selected from the outpatient and inpatient departments of the institution and divided in four study groups, each of 30 subjects as follows.

Group: 1 - Healthy non-smoking individuals as control. They were non-smokers with no history of smoking or any major illness.

Group: 2 - Healthy smokers. They had been smoking since at least 10 years with a minimum of 1 pack per day i.e. smokers having smoking history of at least 10 pack years. Here, one "pack year" is 20 cigarettes/80 beedies smoked /day for one year. (4 beedies are equivalent to 1 cigarette). [9]

Group: 3 - Non-smoking patients suffering from COPD since 5 years or more.

Group: 4 - Smoking patients suffering from COPD since 5 years or more.

The diagnosis and staging of COPD was done by PFTs using GOLD criteria. [8]

Exclusion criteria

- Subjects suffering from other major diseases like diabetes, hypertension, or cardiac disorders.
- Subjects having cancer or undergone lung surgery (e.g. lung reduction, lung transplant).
- Subjects suffering from other respiratory disorders like asthma, lung cancer, sarcoidosis, tuberculosis, lung fibrosis, pneumoconiosis etc.
- Terminally ill patients with COPD.
- Serious, uncontrolled disease (including serious psychological disorders) likely to interfere with the study or impact on safety of the subject.

Pulmonary function tests (PFTs): PFTs were carried out in all study groups. Two consecutive sputum samples were tested before PFT to rule out tuberculosis. PFTs were recorded using "Medgraphics U.S.A Body Plethysmograph, Elite DX Model No-830001-005". Volume calibration was done at three litres and

temperature calibration at room temperature to give values at BTPS. The parameters used were FVC, FEV₁ and FEV₁/FVC.

Estimation of Serum Superoxide Dismutase

Superoxide dismutase was assayed in all the study groups by the method devised by Marklund S, Marklund G modified by Nandi and Chatterjee. [10, 11] Venous blood samples were collected from all the subjects. In case of smokers, samples were collected at least 12 hours after last smoke to avoid acute changes in SOD levels due to smoke.

Principle

Pyrogallol autooxidises rapidly in aqueous or alkaline medium solution and this has been employed for the estimation of superoxide dismutase. SOD inhibits the auto oxidation of pyrogallol. This principle was employed in a rapid and convenient method for the determination of the enzyme concentration.

Reagents

1. Tris Buffer

50 ml of Tris buffer (containing 50 mM of Tris buffer and 1 mM of EDTA) was prepared. To this, 50 ml HCL was added to adjust the pH at 8.5 and volume was made up to 100 ml.

2. Pyrogallol (20 mM concentration)

25 mg of pyrogallol was dissolved in 10 ml of distilled water.

Procedure

For Control

To 2.9 ml of Tris buffer, 0.1 ml of pyrogallol solution was added, mixed and reading was taken at 420 nm, exactly after 1 minute 30 seconds and 3 minutes 30 seconds. The absorbance per two minutes was recorded and the concentration of pyrogallol was adjusted (by diluting the pyrogallol solution) so that the rate of change of absorbance per minute was approximately 0.020 – 0.023 nm.

For Sample

To 2.8 ml of Tris buffer, 0.1 ml of serum sample was added, mixed and started the reaction by adding 0.1 ml of adjusted pyrogallol solution (as per control). It was read at 420 nm exactly after 1 minute 30 seconds and 3 minutes 30 seconds and absorbance per 2 minutes was recorded.

Calculations

Absorbance reading of control - A

Absorbance reading of sample - B

Units of SOD/3 ml of assay mixture = $[(A-B) / (A \times 50)] \times 100$

Unit $\times 10$ = Units /ml of sample solution.

Definition of Unit

One unit of superoxide dismutase is described as the amount of enzyme required to cause 50 % inhibition of pyrogallol auto oxidation per 3 ml assay mixture.

Normal range

SOD in serum is 2.93-3.71 units/ml.

The data was analysed by ANOVA, Pearson's correlation coefficient and Unpaired t test using Graph Pad Prism, version- 5 statistical software.

Result

Table-1 Comparison of the demographic data of the study groups

Parameter	Control	Healthy smokers	Non-Smoking patients with COPD	Smoking patients with COPD	P-value (One way ANOVA)
Age (years) (Mean ± SD)	53.87 ± 8.85	53.17 ± 7.39	54.47 ± 8.23	55.10 ± 7.95	0.8176 (NS)
Weight (Kg) (Mean ± SD)	60.53 ± 8.94	60.77 ± 7.55	58.73 ± 10.01	58.50 ± 12.91	0.7427 (NS)
Height (m) (Mean ± SD)	1.62 ± 0.07	1.62 ± 0.07	1.58 ± 0.06	1.59 ± 0.07	0.750 (NS)
BMI (Mean ± SD)	23.14 ± 3.50	23.18 ± 3.11	23.48 ± 4.37	22.98 ± 4.24	0.9661 (NS)

NS: Statistically not significant

Table-2 Comparison of serum SOD levels in control and healthy smokers

Parameter	Control	Healthy smokers	P-value
Serum SOD (Mean ±SD)	3.01 ± 0.23	2.50 ± 0.45	< 0.001 (HS)

HS: Statistically highly significant
SD: Standard Deviation

Table-3 Comparison of serum SOD levels in control and non-smoking patients with COPD

Parameter	Control	Non-smoking patients with COPD	P-value
Serum SOD (Mean ± SD)	3.01 ± 0.23	2.36 ± 0.49	< 0.001 (HS)

HS: Statistically highly significant

Table-4 Comparison of serum SOD levels in control and smoking patients with COPD

Parameter	Control	Smoking patients with COPD	P-value
Serum SOD (Mean ±SD)	3.01 ± 0.23	2.23 ± 0.43	< 0.001 (HS)

Table-5 Comparison of serum SOD levels in healthy smokers and non- smoking patients with COPD

Parameter	Healthy smokers	Non- smoking patients with COPD	P-value
Serum SOD (U/ml) (Mean ± SD)	2.50 ± 0.45	2.36 ± 0.49	0.2691 (NS)

Table-6 Comparison of serum SOD levels in healthy smokers and smoking patients with COPD

Parameter	Healthy smokers	Smoking patients with COPD	P-value
Serum SOD (U/ml) (Mean ± SD)	2.50 ± 0.45	2.23 ± 0.43	0.0218 (S)

S: Statistically significant

Table-7 Comparison of serum SOD levels in non- smoking patients with COPD and smoking patients with COPD

Parameter	Non- smoking patients with COPD	Smoking patients with COPD	P-value
Serum SOD (U/ml) (Mean ± SD)	2.36 ± 0.49	2.23 ± 0.43	0.2747 (NS)

Table-8 Correlation of serum SOD levels with FEV₁ % predicted in non-smoking patients with COPD

FEV ₁ % Predicted (Mean ± SD)	Serum SOD (U/ml) (Mean ± SD)	Pearson's correlation coefficient
74.07 ± 25.14	2.63 ± 0.49	0.484

Table-9 Correlation of serum SOD levels with FEV₁ % predicted in smoking patients with COPD

FEV ₁ % Predicted (Mean ± SD)	Serum SOD (U/ml) (Mean ± SD)	Pearson's correlation coefficient
48.77 ± 20.95	2.24 ± 0.43	0.676

Discussion

Oxidative stress is the potential mechanism contributing to cigarette smoke related lung diseases like COPD that overwhelms the primary antioxidant defence of the lung. One of the crucial antioxidant defenses of the lung is SODs, which are the only enzyme family with activity against superoxide radicals. [7] It catalyzes the dismutation of superoxide radicals (O_2^-) into O_2 and H_2O_2 . A major question in the pathogenesis of COPD is why this disease develops only in a modest fraction (15–20%) of smokers. [3] One possibility is the variable induction of the primary antioxidant defence in smokers' lungs. In the present study SOD levels were decreased significantly in both non-smoking patients with COPD as well as smoking patients with COPD, but there was statistically no significant difference of SOD levels between these two groups. So, it can be interpreted that, smoking is not the only factor to cause aetiopathology of COPD and there are some other factors responsible for producing oxidative stress which lead to COPD. Other factors may be in the form of interactions among infection, inflammation, protease/antiprotease imbalance, oxidative stress, environmental pollution and apoptosis. Also, genetic factors, diet can affect the pathogenesis of COPD. Genetic polymorphisms are likely explanations for such differences in the pathogenesis of COPD. However, despite the fact that COPD is a leading cause of death in developed countries and has been extensively studied, the only well-established genetic risk factor for COPD is α 1-antitrypsin deficiency. However, α 1-antitrypsin deficiency is responsible for only 1–2% of all cases of COPD. Polymorphisms in other genes proposed to be linked to susceptibility to COPD include the promoter of the tumor necrosis factor- α , aromatic-inducible cytochrome P450 subfamily 1 polypeptide 1, cystic fibrosis transmembrane conductance regulator, α 2-macroglobulin, α 1-antichymotrypsin, and endothelial nitric oxide synthase, but definitive links with these genes and COPD have not been established. [12, 13] Following are some studies in agreement with the present study.

Kim SH et al (2003) found that the extracellular superoxide dismutase activities were lower in smokers than in non-smokers. [13]

Raghnath R. Rai and Madhavi S. Phadke (2006) found that in patients with COPD, activity of enzyme SOD was significantly decreased than that of control group. [14]

M K Daga et al (2003) found that, in the smokers with COPD, the serum SOD levels were 30.9% lower ($P < 0.001$) in smokers compared to controls. [15]

Similarly, Kondo T et al (1994) found that smoking in elderly men reduces antioxidants in alveolar macrophages with increase in the level of oxygen radical species. There was decrease in the levels of SOD in alveolar macrophages from elderly chronic smokers. [16]

Jain A, et al (2009) studied antioxidant status and smoking habits in individuals who had been smoking beedi or cigarettes for more than one year. Erythrocyte SOD was significantly lower in cigarette smokers and beedi smokers as compared to non-smokers due to the utilization of these antioxidants for the scavenging of free radical generation. Additionally, this study also showed that beedi smokers have more oxidative stress than the cigarette smokers. This can be accounted on the basis of excess of carbon monoxide, tar and other toxic constituents present in the smoke of the beedi. Beedis contain higher levels of steam volatile phenol, hydrogen cyanide and benzopyrene along with higher level of particulate matter and nicotine. [17]

Thus, the present study and the above studies may point towards the fact that increased production of free radicals in the patients with COPD and smokers leads to increased consumption of SOD leading to decrease in the SOD levels.

A study by C Blake Gilks et al (1998) explains that there was initial up-regulation and then rapid down-regulation of Mn SOD expression in the initially low-expressing bronchiolar epithelial cells in response to cigarette smoke. This could represent an acute protective mechanism against oxidant damage. [18]

There are a few studies like a study by Neil R. Hackett et al (2003) which have found that there was no change in the SOD levels in smokers and COPD patients. [13]

Here, the possible reasons for such discrepancies could be related to differences in inter-individual variations in antioxidant capacity as a result of different populations and also differences in methodologies between studies.

There are very few studies regarding correlation of severity of obstruction i.e. pulmonary function and oxidant or antioxidant levels. Most of such studies showed inconsistent results. Some of these old studies include, study by Petruzzelli et al. (1990) who found inverse relation between malondialdehyde (MDA) and degree of small airway obstruction. [19]

A study by Kluchova et al (2007) showed the correlation between SOD and severity of COPD. Even in that study also, there was no difference observed in the SOD activities in the subjects with severity of COPD. [20] So, the present study is an attempt to find out this relationship and in this study, it was observed that, there was progressive decrease in the levels of serum SOD in accordance with increase in the severity of the disease i.e. serum SOD levels were positively correlated with pulmonary function.

There are no previous studies found which are confined to investigating SOD levels in only the non-smoking patients with COPD, this study is an attempt to reveal the findings in this group, in which it was found that there was decrease in serum SOD levels in non-smoking patients with COPD as compared to control due to consumption of the antioxidants in scavenging the free radicals.

In the present study, the serum SOD levels were significantly lower in smoking patients with COPD than that in the healthy smokers. This denotes a possibility that, there may be a decrease in SOD levels due to COPD itself.

Thus to conclude, it can be said that, as there is intense diversity regarding aetiopathogenesis of COPD and its correlation with smoking, this study was an attempt to establish a relationship among oxidative stress, COPD and smoking. Even though it was interpreted from the present study that, smoking is not the only causative factor for COPD, it is sure that oxidative stress is one of the associated factors in COPD, either as a cause or as a result of COPD itself.

As the role of oxidative stress in the pathogenesis is well established, further detailed studies are required to combat this stress, especially by using the easily available antioxidants, either dietary or in therapeutic forms. Detecting changes in the levels of SOD can help in earlier prediction of COPD which may be used to halt further progression of the disease or follow up of its treatment and to save the cost of its management.

Future studies with larger sample size inclusive of different age groups are needed to study the pathogenesis of COPD in non-smokers for better understanding of its aetiopathogenesis.

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