Research Paper

SERUM EXTRA CELLULAR SUPEROXIDE DISMUTASE AND ASCORBIC ACID LEVELS IN SMOKER AND NON-SMOKER CHRONIC OBSTRUCTIVE PULMONARY DISEASE PATIENTS

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Background: Chronic Obstructive Pulmonary Disease (COPD) is a health problem with increasing severity, as exposure to risk factors such as cigarette smoke, pollution is inevitable. Oxidant-antioxidant imbalance cause oxidative burden leading to lung tissue damage. Presently, diagnosis of COPD is based on impaired lung function tests. Aims and Objectives: This study was aimed to measure serum levels of extra-cellular Superoxide Dismutase (ecSOD), a major antioxidant enzyme in pulmonary and extra pulmonary compartment and versatile antioxidant ascorbic acid in various stages of COPD and to correlate them with BMI and smoking status. Methods: The study involved 100 stable COPD patients grouped in to four stages based on GOLD criterion (FEV₁ % predicted). Results: Serum ecSOD and ascorbic acid levels show decreasing trend across the stages of COPD. Mean ecSOD is higher while mean ascorbic acid level is lower in active smokers than non-smokers and ex-smokers. Ascorbic acid has significant positive correlation (r=0.4, p<0.05) while ecSOD shows moderate positive correlation (r=0.5, p<0.05) with FEV₁ % predicted. Conclusion: Raised ecSOD and lower ascorbic acid levels, indicate excess of oxidative stress in active smokers and they have shown decreasing trend across the stages of COPD. ecSOD may serve as potential biomarkers in COPD pathophysiology. Dietary supplementation of ascorbic acid to COPD patients is strongly recommended.

Keywords: COPD, Serum ascorbic acid, Serum ecSOD

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) has become global health problem now a day. Inevitable exposure to risk factors like pollutants, cigarette smoke, fast and complicated lifestyle are some of the risk factors which appears to be the main culprits in pathogenesis of COPD. Recent studies showed prevalence of COPD as

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4.1 and prevalence ratio 2.65:1 in smokers and non-smokers in India, emphasizing COPD as major upcoming health problem with increasing severity in India (Jindal et al., 2006; Viegi et al., 2001).

In fact COPD is a group of diseases with common clinical findings such as productive cough, dyspnoea. Characteristically it has progressive airflow limitation and abnormal inflammatory response of lung to noxious particles or gases. The airflow limitation is not fully reversible and there are no treatment modalities at present to prevent progressive airflow limitation. This has renewed the interest to study the underlying cellular and biochemical mechanisms in etiopathogenesis of COPD (Barnes, 2004; Jadhav et al., 2013).

Pulmonary function tests primarily confirm the diagnosis of COPD. Forced expiratory volume in one second (FEV₁) and Forced Vital Capacity (FVC) are the key parameters in spirometry. The most sensitive index for early diagnosis of COPD is decreased FEV₁/FVC ratio (Reilly et al., 2005). GOLD guidelines propose a classification into four stages based on functional impairment only. This classification overlooks the inflammatory markers and cellular changes in lung and peripheral tissues (Rufino and Lapa de-Silva, 2006). History of smoke exposure, whether present or past, is one of the most important factors in development of COPD. It is seen that 90% COPD patients give history of smoking (recent or remote) out of which only 10-15% develop COPD. This may be due to variable individual susceptibility for genetic and environmental factors for development of COPD in patients (Kelly, 2005).

The baseline event that activates the inflammatory cells in lower respiratory tract is cigarette smoke exposure. It recruits inflammatory cells to induce local inflammatory response and repair mechanisms (Ryrfeldt et al., 1993; Jadhav et al., 2013). These responses cause increase in free radical production leading to excess of oxidative burden in pulmonary tract as well as in systemic circulation (Oudijk et al., 2003; VanEeden et al., 2005). Various antioxidant mechanisms neutralize the excessive oxidative stress and protect the tissues from damage. Ascorbic acid is the principle antioxidant molecule of aqueous phase. It also recycles the antioxidant capacity of oxidized vitamin E which is the integral component of surfactant (Kolleck et al., 2002; Schunemann et al., 2001). Extra-cellular Superoxide Dismutase (ecSOD) is one of the major anti-oxidant enzymes in pulmonary tissue as well as in systemic circulation which provides protection from excessive oxidative stress (Gey, 1993; Bowler and Crapo, 2002). The aim of present study was to assess serum Ascorbic acid and serum ecSOD levels in COPD patients and to find out association between these parameters and pulmonary function tests, if any, and to compare the results in smoker and non-smoker COPD patients.

**MATERIALS AND METHODS**

Present study is a cross sectional analysis, performed in Department of Biochemistry, Government Medical College, Aurangabad in collaboration with Shree nursing home and Department of Medicine Government Medical College, Aurangabad 100 diagnosed patients of COPD attending Medicine OPD were enrolled in the study, following institutional ethical committee permission and guidelines. Patients with acute infections like Upper respiratory tract infections, inflammations and other chronic diseases like
exacerbation of COPD, congestive cardiac failure, and anemia were excluded. Stable COPD patients (smokers as well as non-smokers) from urban area were randomly included in study, as they attended Medicine OPD.

The smoking status of subjects is taken as ‘Active smoker’ when subject is currently smoking daily at least one cigarette. It is considered as ‘Ex-smoker’ when the subject smoked last time for about a minimum of six months or more. ‘Non-smoker’ is that subject who never has past history of smoking of tobacco in any other mode/form and even never had passive smoking situations.

Pulmonary function tests were carried out in the morning hours which involved measurement of FEV$_1$ % predicted, FVC. Patients were grouped into four stages based upon ‘FEV$_1$ % predicted’ according to GOLD guidelines (Gold, 2007). It is stage I when the value is up to 80% and it is stage II when it varies between 50-80% while in stage III it varies between 30-50%. It is stage IV when the value falls below 30% of predicted value or is equal to 50% while signs of right heart failure or respiratory failure are positive. In all stages, FEV$_1$/FVC ratio is <0.7 (Table 1). Further patients were divided into three groups according to their smoking status, and again grouped into four stages based on GOLD criterion. BMI of patients was calculated, by using formula height (cm$^2$) divided by weight (kg). Seven mL of venous blood was collected from each patient at random divided in two parts and transferred to citrate bulb for Ascorbic acid and to plain bulb for serum ecSOD estimation.

Estimation of Ascorbic acid was done manually by colorimetric method using acid phosphotungustate on spectrophotometer at 660 nm (Ayekygw, 1978). Estimation of ecSOD was done manually by modified kinetic method (Nandi and Chaterjee, 1988). It is based on colorimetric measurement of inhibition of autoxidation of pyrogallol at pH 8.5. All the readings were taken on Thermo-Spectronic (UK) spectrophotometer at 420 nm.

Statistical analysis was done using graph pad and SPSS version 17 software. ANOVA test was employed to find out significance of the difference in the mean values of biochemical parameters across various stages of COPD and various groups according to smoking status.

**OBSERVATIONS AND RESULTS**

Distribution of COPD patients into four stages according to the smoking status is shown in Table 2. Table 3 shows values of serum ecSOD and Ascorbic acid across the COPD stages. Ascorbic acid show fall in mean values up to stage III, which is statistically significant (p<0.05) in comparison to stage I (Figure 1), thereafter it is unchanged. Serum ecSOD levels show steady decline in mean values which is statistically significant (p<0.05) as compared to stage I (Figure 1). It can also be seen from Table 3 that BMI decreases as stage advances.

When the biochemical parameters were analyzed for association with clinical parameters, it is found that ecSOD has moderate positive correlation with FEV1% pred. (r=0.5, p<0.05), while there was no significant correlation between ecSOD and the BMI of patients. It was interesting to note that Ascorbic acid level has moderate positive correlation with FEV1% pred. (r=0.4), which is statistically significant (p<0.05). BMI did not show any significant correlation with Ascorbic acid.
Table 1: GOLD Classification of COPD Patients

<table>
<thead>
<tr>
<th>GOLD Stage</th>
<th>Severity</th>
<th>Spirometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>At risk</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Mild</td>
<td>FEV$_1$/FVC &lt; 0.7 and FEV$_1$ up to 80% predicted</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>FEV$_1$/FVC &lt; 0.7 and FEV$_1$ = 50-80 % predicted</td>
</tr>
<tr>
<td>3</td>
<td>Severe</td>
<td>FEV$_1$/FVC &lt; 0.7 and FEV$_1$ = 30-50% predicted</td>
</tr>
<tr>
<td>4</td>
<td>Very severe</td>
<td>FEV$_1$/FVC &lt; 0.7 and FEV$_1$ &lt; 30% predicted or FEV$_1$ &lt; 50% predicted with respiratory failure or signs of right heart failure.</td>
</tr>
</tbody>
</table>


Table 2: Distribution of the COPD Patients in Various COPD Stages

<table>
<thead>
<tr>
<th>FEV$_1$% pred*</th>
<th>COPD Stage I 78 ± 7.127</th>
<th>COPD Stage II 59.97 ± 9.16</th>
<th>COPD Stage III 40.67 ± 5.90</th>
<th>COPD Stage IV 25.05 ± 6.69</th>
<th>Total Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smokers</td>
<td>5</td>
<td>12</td>
<td>18</td>
<td>4</td>
<td>39</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>1</td>
<td>16</td>
<td>19</td>
<td>17</td>
<td>53</td>
</tr>
<tr>
<td>Active smokers</td>
<td>0</td>
<td>03</td>
<td>04</td>
<td>01</td>
<td>08</td>
</tr>
<tr>
<td>Total Patients</td>
<td>6</td>
<td>31</td>
<td>41</td>
<td>22</td>
<td>100</td>
</tr>
</tbody>
</table>

Note: *Figures are mean ± SD values.

Table 3: ecSOD, Ascorbic Acid, BMI in COPD Patients in Relation with COPD Stages

<table>
<thead>
<tr>
<th>Reference Value</th>
<th>Stage 1(n=6)</th>
<th>Stage 2(n=31)</th>
<th>Stage 3(n=41)</th>
<th>Stage 4(n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/ml)</td>
<td>2.93 ± 3.7</td>
<td>3.31 ± 0.32</td>
<td>2.49 ± 0.25*</td>
<td>2.10 ± 0.40*</td>
</tr>
<tr>
<td>Ascorbic acid (mg %)</td>
<td>0.2 - 0.4</td>
<td>0.37 ± 0.02</td>
<td>0.32 ± 0.06*</td>
<td>0.24 ± 0.07*</td>
</tr>
<tr>
<td>BMI</td>
<td>18.5 – 24.99</td>
<td>22.19 ± 5.13</td>
<td>22.39 ± 4.11</td>
<td>21.06 ± 5.53</td>
</tr>
</tbody>
</table>

Note: Figures are mean ± SD values. * P value (<0.05) statistically significant.

It is observed that the mean values of serum ecSOD values are higher in active smokers, when compared with ex-smokers and non-smokers and were statistically significant (p<0.05) (Table 4). Active smokers show significantly low Ascorbic acid values, when compared with ex-smokers and non-smokers (p<0.05). In this study, we found that the mean values of BMI were significantly lower in ex-smokers when compared with non-smokers (p<0.05).

DISCUSSION

Delicate balance between oxidative stress and antioxidant mechanisms is essential for normal functioning of organ systems. Lungs have direct and continuous exposure to high oxygen tension. Thus pulmonary tissue needs strong antioxidant
back up (VanEeden et al., 2005; Kluchová et al., 2007; Rai and Phadke, 2006). ecSOD is a unique enzyme having prominent expression around airways and airway smooth muscle as well as in systemic circulation (Bowler and Crapo, 2002). ecSOD catalyzes the dismutation of two superoxide radicals into hydrogen peroxide and oxygen. Hydrogen peroxide can be easily converted to water and molecular oxygen by ubiquitous catalase enzyme. Along with ecSOD, Ascorbic acid is major aqueous antioxidant playing vital role in dealing with oxidative stress in respiratory epithelial lining fluid of the lung. It is an important antioxidant biomolecule both within cells and in the plasma. It not only prevents the leukocyte recruitment that initiates the inflammatory response but also intercept the oxidative free radicals at aqueous phase as a protection to lipid peroxidation (Lehr et al., 1994; Schunemann, 2001).

This study finds significant positive correlation between serum ecSOD levels and stages of COPD (r=0.5, p<0.05). The mean value of ecSOD was observed to fall across the stages of COPD (Table 3). It is also reported that the mean values of serum SOD levels were low in COPD patients as compared to controls (Rai and Phadke, 2006). Decrease of SOD levels in COPD patients is invariably interpreted in several reports

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-smokers (n=39)</th>
<th>Ex-smokers (n=53)</th>
<th>Active Smokers (n=08)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (units /ml)</td>
<td>2.35 ± 0.54</td>
<td>2.21 ± 0.41</td>
<td>2.56 ± 0.48*</td>
</tr>
<tr>
<td>Ascorbic acid (mg %)</td>
<td>0.29 ± 0.07</td>
<td>0.28 ± 0.07</td>
<td>0.21 ± 0.07*</td>
</tr>
<tr>
<td>BMI</td>
<td>23.03 ± 4.41</td>
<td>19.93 ± 3.72*</td>
<td>20.94 ± 4.3*</td>
</tr>
</tbody>
</table>

Note: Figures are mean ± SD values. * P value (<0.05) statistically significant.
as due to increased oxidative stress. The fall in the mean SOD levels across the stages of COPD certainly reflects the decline of antioxidants with dominance of pro-oxidants.

The mean values of serum ecSOD levels are higher in active smokers when compared with non-smokers and ex-smokers. Similar finding was reported in a study that found raised SOD levels in active smokers (Rahman and MacNee, 1996). On similar lines other studies found increased RBC SOD levels in smokers as compared to controls (Sayyad et al., 2008). This indicates that active smokers show compensatory enhanced antioxidant enzymatic activity to ameliorate the potential damage from excess oxidative stress. Increasing load of free radicals formed as a consequence of cigarette smoking might be inducing more expression of ecSOD. It is reported earlier that free radicals can induce expression of SOD at genetic level (Kobzik et al., 1993). Non-smokers who are not exposed to smoke and ex-smokers, who have lost contact with smoke for long period of time, did not show higher levels than the controls. Thus our observations strongly supports our contention that free radicals secondary to smoking are inducing ecSOD expression in active smokers which result in higher SOD levels both intra as well as extra cellular compartments.

In the present study, it is observed that the mean values of Ascorbic acid levels fall across the stages of COPD which is statistically significant (p<0.05). Similar findings are reported in earlier studies (Rai and Phadke, 2006; Calikoglu et al., 2002). Britton et al. reported a cross-sectional association between FEV1 and intake of Ascorbic acid. Higher intake of Ascorbic acid was found to be associated with a lower rate of decline of FEV1 (Britton et al., 1995). Schwartz and Weiss found a beneficial association between both dietary and serum Ascorbic acid and bronchitis symptoms (Schwartz and Weiss, 1990).

Thus, from published reports and available information on role of ascorbic acid in COPD, it is likely that there is shift of Ascorbic acid from serum to pulmonary tract so as to ameliorate the excessive oxidative stress in respiratory epithelial lining fluid (Schunemann et al., 2001). This may be one of the reasons for the lower ascorbic acid levels across the COPD stages. Thus in stage IV, the available endogenous antioxidant cover, which includes SOD, becomes inadequate to balance the pro-oxidant load and starts to decline. The increased expression of SOD also gradually falls as the exposure to smoke becomes higher and longer duration as seen in stage IV COPD patients. The consequence of these molecular changes alters the cellular pathology and eventually becomes visible as clinical manifestation seen in late stage COPD. From present findings and with support of earlier published reports (Britton et al, 1995, Schwartz and Weiss, 1990), it is suggested that dietary supplementation of ascorbic acid in adequate doses to COPD patients should be promoted as auxiliary regular incentive therapy. It must be included in the mainline therapeutic regime. This strategy would at least slow down the rate of progression of airflow limitation in the patients of COPD.

This study observed a fall in BMI as the COPD stage advances (Table 3). This observation indicates only the systemic involvement prevalent in COPD. There may not be any causal relationship of BMI and changes in other
biochemical parameters studied in the COPD patients.

It appears that the biochemical parameters such as serum ecSOD and Ascorbic acid levels have some role in pathophysiology of COPD. The finding of this study has positive implication in the clinical management of COPD patients. These may also help in assessing disease severity and/or prognosis in response to therapeutic interventions in COPD. Dietary supplementation of ascorbic acid along with routine drug therapeutic regimen should give more desired benefits to COPD patients.

CONCLUSION

The mean levels of ecSOD were found to be lower as the stage of COPD advance, representing the depletion of antioxidant mechanisms. The levels were significantly higher in current smokers as compared to ex-smokers and non smokers. This is an adaptive response to ameliorate excessive oxidative stress present in active smokers. The mean values of serum ascorbic acid decrease across the COPD stages. Significantly lower serum mean levels of ascorbic acid were found in active smokers as compared to ex-smokers and non-smokers. The present study clearly accentuates the importance of ecSOD and Ascorbic acid as one of the protective mechanisms to combat excess oxidative stress in COPD. A dietary supplement of ascorbic acid along with mainline therapy is strongly recommended for maximum benefits to COPD patients.

CONFLICTS OF INTEREST

We declare that there are no any conflicts of interests.

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REFERENCES


