COMPARATIVE STUDY OF PULMONARY FUNCTIONS AND OXIDATIVE STRESS IN SMOKERS AND NON–SMOKERS

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(Received on November 5, 2011)

Abstract: Chronic Obstructive Pulmonary Disorder (COPD) is projected to rank third leading cause of deaths by 2030 as per WHO. COPD is a multi-etiological disease. The airflow dysfunction is usually progressive, associated with an abnormal inflammatory response of the lungs to noxious particles or gasses. As the lung is exposed to high levels of oxygen, it is more susceptible to oxidants mediated injury. Gender based differences are identifiable risk factors. Smoking is found to be a major risk factor in the causation of COPD resulting in oxidative stress. The aim of the present study is to evaluate the oxidant antioxidant imbalance in healthy non smoker controls and smokers with COPD. A total of 60 control (healthy non smokers) and 121 smokers having COPD were studied. The mean age is more in smoker group as compared to healthy controls, which identifies advancing age as a risk factor for COPD. The mean BMI and weight of smoker group is reduced as compared to control group. GOLD 2008 criteria was used to assess lung functions. Lung functions namely FEV1, FVC, FEV1/FVC% and FEV1% Predicted showed significant reduction in smoker group as compared to healthy non smoker controls. MDA in control and smoker group (1.09±0.09 and 1.41±0.23 nmol/ml respectively) showed significant changes (P<0.001). Our results also demonstrate significant reduction in anti oxidant enzymes namely SOD (units/mg of serum protein), Catalase (units/mg of serum protein) and GPX (nmol of NADPH oxidized/min/mg of serum protein) in smoker group as compared to healthy controls. On the basis of study it is concluded that smoking, gender and oxidant antioxidant imbalance are identifiable risk factors in COPD.

Key words: smoking gender MDA (Malondialdehyde) SOD (Superoxide Dismutase) catalase GPX (Glutathione Peroxidase)

INTRODUCTION

Chronic Obstructive Pulmonary Disorder (COPD) is projected to rank third leading cause of deaths by 2030 (1). In a large multicentric study in India the prevalence

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of chronic bronchitis was found to be 4.1% in adults above 35 years of age with a male female ratio of 1.56 and smoker non smoker ratio of 2.65 (2). ICMR reported 4.29% males and 2.7% females suffer from asthma and COPD (3). COPD is a multi-etiological disease. The airflow dysfunction is usually progressive, associated with an abnormal inflammatory response of the lungs to noxious particles or gases. The major risk factors, contributing to COPD, can be divided into (i) External, and (ii) Internal Factors. The most common Risk Factor, contributing to COPD, is tobacco smoking (4, 5, 6, 7). Oxidative injury, due to exposure to oxidants from diverse sources including air pollution, cigarette smoke, and endogenous oxidants, resulting from a lack of antioxidants in the body, may at least, in part, be related to reduced FEV1; and, thus, they contribute to airflow-obstruction. Lundback B et al, reported that at least 50 per cent of smokers develop COPD (8). Other literature have reported 15-20% of smokers develop COPD (9, 10). The crucial factor seems to be the amount smoked and also the extent of inhalation (11). National Health Family Survey (NFHS-3) have reported 32.7% males and 1.4% females smoke between age groups 15-49 years, overall smoking increases with advancing age (12). By 2020 India is projected to contribute 18% of tobacco related deaths globally (13). The correlation is so strong and positive that the term “tobaccosis” and “smokers’ lung” have been proposed and are in use as alternative names of the disease (14, 15, 16).

Aim and Objectives: The aim of the present study is to assess the pulmonary functions and oxidant anti-oxidant imbalance in healthy non smoker controls and smokers with COPD.

**MATERIAL AND METHODS**

The patients included in the present Study, were selected from Out-Patients Department (OPD), Emergency Ward, and Indoor Patients, admitted in the Ward for Tuberculosis and Chest Diseases, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh. Institutional Ethics Committee clearance was taken. Healthy subjects serving as controls were selected from the Institution.

**Selection criteria**

Subjects over 18 years of age with no history of Bronchial Asthma, Diabetes Mellitus, Hyper-tension, Lung Cancer, Cardiovascular and Renal Diseases, and other diseases in which oxidative stress has been documented to be a causative factor, were included in the study. Healthy controls were non smokers and with no exposure to biomass fuel. Patients with history of exposure to risk factors for COPD, particularly smoking and/or exposure to smoke from biomass fuel and history of dyspnea and chronic cough, or sputum production on most days for at least three months of the two consecutive years were included in the study. Subjects taking antioxidants were excluded.

**Pulmonary function tests**

PFT was performed using the Mir Spiro Lab II Spirometer. Measurement of Forced Vital Capacity and Forced Expiratory Volume was done in the First Second. The FEV1/FVC is calculated using the Maximum FEV1 and FVC from the technically acceptable, though not (necessarily) from the same
Curves. The Data are obtainable from the printer, attached to the Spirometer.

Estimation of antioxidant enzymes

Under aseptic conditions and with prior consent of the subjects, 5 ml of blood sample was drawn from the peripheral vein from the subjects of both the sexes. It was centrifuged at 3000 rpm for fifteen minutes. The Serum was subjected to estimation of MDA and antioxidants namely Catalase, Glutathione, Peroxidase and Superoxide Dismutase. Catalase was estimated by the method, adopted by Aebi (17). The activity was expressed as units per mg of Serum Protein. Glutathione Peroxidase estimation was done by the technique adopted by Paglia and Valentine (18). Enzymatic activity is expressed as nanomole of NADPH Oxidized per minute per milligram Serum Protein. Superoxide Dismutase was estimated by the method, adapted by McCord and Fridovich (19). Enzyme activity was expressed as units per milligram of Serum Protein.

Protein content of Serum was measured by the method of Lowry et al (20).

Estimation of free radicals

Free radicals were estimated by the method adopted by Philpot (21). MDA levels expressed as nmol/ml.

Statistical analysis

Statistical package for social science (SPSS 12.0) is used for statistical analysis. The results were expressed as Mean±Standard Deviation (S.D). Unpaired t test was applied to analyze the statistical significance of change in the Antioxidants levels and MDA between Controls and smokers with COPD. Pulmonary Function Tests parameters, were also statistically analyzed in the same way. P value <0.05 was taken as significant.

RESULTS AND OBSERVATIONS

The Mean Age of the subjects in Control and Case group was 38.37±13.29 years and 43.15±13.04 years respectively. The number and percentage of male and female subjects in non smokers and smokers was 46(76.67%), 14(33.33%) and 80(66.11%), 41(33.89%) respectively. The mean weight of smokers was lower (49.57±8.50 kg) as compared to non smokers (55.73±8.28 kg). The Body Mass Index (BMI) in smokers was lower(18.88±3.14 kg/m²) as compared to non smokers (20.82±3.05 kg/m²). The Pulmonary Function Tests namely FVC (Liters), FEV1 (Liters), FEV1/FVC % and FEV1% Predicted were significantly (P<0.001) lower in smokers as compared to Control group (non smokers). The serum levels of Malondialdehyde (nmol/ml) was significantly higher (P<0.001) in smokers (1.41±0.23) as compared to non smokers (1.09±0.09). The serum levels of anti oxidant enzymes namely SOD (units/mgm serum protein), Catalase (units/mgm serum protein) and GPX(nmol NADPH

<table>
<thead>
<tr>
<th>Parametre</th>
<th>Healthy non-smoker controls (n=60)</th>
<th>Smokers with COPD (n=121)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38.37±13.29</td>
<td>43.15±13.04</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 46</td>
<td>Female 14</td>
</tr>
<tr>
<td>Height (cms)</td>
<td>163.69±7.30</td>
<td>162.20±7.88</td>
</tr>
<tr>
<td>Weight (kg)*</td>
<td>55.73±8.28</td>
<td>49.57±8.50</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>20.82±3.05</td>
<td>18.88±3.14</td>
</tr>
</tbody>
</table>
oxidized/min/mgm of serum protein) were significantly lower in smokers as compared to non smokers. The results and measured parameters are shown in Table I, II and III respectively.

**TABLE II :** Pulmonary function tests. *Significant at P<0.05.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy non-smoker control (n=60)</th>
<th>Smokers with COPD (n=121)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC (liters)</td>
<td>3.52±0.65</td>
<td>2.80±1.15</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>FEV1 (liters)</td>
<td>2.91±0.56</td>
<td>1.63±0.79</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>FEV1/FVC %</td>
<td>82.65±7.13</td>
<td>56.65±10.04</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>FEV1 % predicted</td>
<td>96.30±0.13</td>
<td>55.73±10.04</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

**TABLE III :** MDA and anti-oxidant enzymes. *Significant at P<0.05.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy non-smoker control (n=60)</th>
<th>Smokers with COPD (n=121)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/ml)</td>
<td>1.09±0.09</td>
<td>1.41±0.23</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SOD (units/mgm of serum protein)</td>
<td>9.10±0.18</td>
<td>9.00±0.14</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Catalase (units/mgm of serum protein)</td>
<td>8.92±0.07</td>
<td>8.88±0.07</td>
<td>0.004*</td>
</tr>
<tr>
<td>GPX (nmol Oxidized NADPH/min/mgm serum protein)</td>
<td>57.21±0.39</td>
<td>51.46±2.77</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Oxygen is important to sustain life. It is relatively non-reactive in ground state. The Cells use Oxygen to generate ATP. During the process, Free Radicals are generated. Theory of Free Radicals has been known over the years (22). Free Radicals by virtue of their having one or more unpaired electrons, are highly unstable and reactive. Lungs are exposed to high levels of environmental oxidants, which together with their large surface area and blood supply, make it susceptible to oxidative stress (23, 24). The reported prevalence of COPD is higher among men than women (25, 26, 27). Sex (male) and increasing age are identified as risk factors in most of the studies from Asia (28, 29). In India, prevalence rates, varying between about 2-22% in men and between 1.2-19% in women, have been shown in different reports[30]. Loganathan R et al, 2006 (31), reported a lower prevalence of COPD in females, as compared to males. In our study male subjects suffering from COPD are higher as compared to females which can be attributed to greater prevalence of smoking among men, as well as cumulative effects of smoking, and other exposures with advancing age. Lower acceptance regarding smoking among females may also contribute to findings. Gender-based differences in Obstructive Airway Diseases occur due to the interaction of sex-dependant genetic factors and also due to socio-cultural differences (32, 33, 34).

In present study mean BMI of cases is lower which can be explained on the basis that COPD, like any other chronic inflammatory disorder, has been associated with increased circulating levels of Tumour Necrotic Factor α (35, 36, 37, 38). There is also increase in the levels of Leptin, which may contribute to weight loss. Low BMI may be attributed to skeletal muscle atrophy and weight Loss (39). Malnutrition and unexplained weight loss are also known with more advanced COPD. Smoking results in weight loss by elevating metabolic rate, reducing metabolic efficiency, or by reducing appetite, which are associated with tobacco use (40).

Although we have not subdivided the
COPD cases on the basis of severity and also inflammatory markers have not been measured which would have further improved the study and given more evidence.

We have found that the smokers have higher oxidant anti oxidant imbalance and reduced lung functions as compared to non smokers. Emphasis is to be given on the fact that smokers having COPD are included in the study and compared with non smokers without COPD. Non Smokers who develop COPD are not included and it's the limitation of the present study.

Cigarette smoke contains more than 6000 Chemical Compounds, and both Free Radicals and Oxidants are present in abundance (41). Smoking induces inflammation and alters repair mechanisms. Sethi JM et al (42), reported that higher the smoking intensity, greater will be the decline in FEV1. A Study conducted by Jindal SK et al (43) found that COPD was diagnosed in 4.1% of the Study Subjects. Smoker to non-smoker ratio of 2.65:1 was found. Studies have shown higher prevalence of COPD in smokers (44, 45). In the NHANES III survey (46), COPD was found to be five times more common in smokers. Pierachille S et al (47), reported a marked reduction in FEV1 and FEV1% in COPD patients, when compared with the healthy controls. The study conducted by Daphne CR et al (48), reported FEV1% decline in the COPD. We have also found decline in lung functions in COPD group as compared to non smokers without COPD which is explained on the basis of interplay of Inflammation, remodeling, bronchospasm, mucus hypersecretion, loss of elastic recoil and increased airway resistance, resulting in progressive reduction in the expiratory airflow. Authors would like to emphasize on the fact that inflammatory markers and PEFR has not been included in the present study.

In present study smokers with COPD have higher MDA levels which probably resulted due to smoking induced radical chain reaction leading to lipid peroxidation of Membrane Phospholipids, altering Cellular Physiology. The lipid peroxides yield a variety of by products including aldehydes. ROS and RNS cause alteration of proteins, chemical fragmentation or increased susceptibility to proteolytic attack, free radicals react with nucleic acid by addition to bases or abstractions of hydrogen atoms from the sugar moiety (24). Though the present study do not include healthy smokers but studies have reported that the plasma levels of MDA are increased in healthy smokers and in patients with COPD (49, 50). A study conducted by Birgul I et al (51), showed that MDA levels are significantly higher in smokers than in non-smokers. Reznick etal (52), reported an increase in Lipid Peroxides after exposure to cigarette smoke.

The tissues are protected against the oxidants by the presence of enzymatic (Catalase, Superoxide dismutase, Glutathione peroxidase) and non-enzymatic antioxidant defense systems (6). The explanation for reduced SOD activity is a possible direct inactivation of the Enzyme by Hydrogen Peroxide or by the Superoxide Anion itself (53, 54).

Altuntas E et al (55), reported an increase in MDA and decrease in catalase in non-smokers and patients with COPD. Similarly increase in MDA and decrease anti oxidant
enzymes have been reported in COPD patients compared to healthy non smokers/controls (56, 57). Liu Ling Yun et al (58), reported an increase in MDA and decreased levels of SOD and GPX in stable and acute exacerbation in COPD Group, as compared to Controls. Nadeem et al (59), reported an increase in MDA levels in COPD patients, as compared to healthy non-smoking controls. They found an increase in levels of SOD, Catalase and GPX in severe COPD patients, as compared to moderate COPD patients. Although in our study the smokers with COPD have not been subdivided on the basis of years of smoking and severity but we have found that MDA levels are higher and antioxidant levels are lower as compared to healthy non smokers which can be attributed both to the fact that free radicals generation is more in smokers and smoking is a major risk factor for COPD.

Conclusion

As lung is exposed to high levels of Oxygen, it is more susceptible to Oxidants mediated injury. Gender based differences are identifiable Risk Factors. Smoking is risk factor in the causation of COPD resulting in oxidative stress enhances inflammation; and, thus the interest in antioxidants as a future mode of treatment for COPD, is widely increasing. Tobacco cessation and awareness should be pursued more aggressively. Further research, both at Molecular and Gross Levels, is required to reduce the risk as well as incidence of COPD which is projected to rank the third leading cause of death globally by 2030.

Limitations of the study: The study is not free from selection bias and years of smoking is not included. COPD is a multi-etiological disease. Not all smokers develop COPD although it is the major risk factor. Similarly, non smokers also develop COPD. Improvement in the study needed is subdivision of COPD patients into smokers and non smokers/ex-smokers.

ACKNOWLEDGEMENTS

Authors are thankful to the supporting staff of the Department of Physiology, TB and Chest Diseases and Biochemistry for helping in innumerable small ways for the completion of study. Express sincere thanks for the participants in the study.

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