Evaluation of neutrophil elastase/ alpha-1-antitrypsin ratio in different stages of chronic obstructive pulmonary disease (COPD) patients

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Abstract

Introduction: Little is known about protease, anti-protease markers in chronic obstructive pulmonary disease (COPD) patients. Objectives: The objective of present study was to identify and try to correlate serum markers of protease and anti-protease with pulmonary functions in different stages of patients with COPD and to determine the ratio of neutrophil elastase/alpha-1-antitrypsin in different stages of COPD patients. Methods: This prospective observational study was carried out in patients with stable COPD. Activities of serum alpha-1-antitrypsin and neutrophil elastase were measured in 220 stable COPD patients and in 60 healthy controls by ELISA method. 220 COPD patients were divided into 4 stages according to severity: stage I, II, III and IV.

Results: An increase in serum neutrophil elastase and neutrophil elastase/alpha-1-antitrypsin ratio was observed in COPD patients with the advancement of the stage. In contrast to that, decreased activity of alpha-1-antitrypsin in serum was observed in different stages of COPD and is correlated positively with lung function parameters.

Conclusion: From these findings we conclude that as the severity increases there is decrease in alpha-1-antitrypsin resulting in concomitant increase in neutrophil elastase activity causing imbalance between protease-antiprotease in COPD patients and this imbalance is associated with impairment of lung function. The neutrophil elastase/ α-1-antitrypsin ratio can tell us the severity of the chronic obstructive pulmonary disease it may be in terms of increased fibrosis of the lung. Though the magnitude of neutrophil elastase/alpha-1-antitrypsin ratio is minute, still it can be a good marker of pulmonary function in term of severity of the COPD.

Keywords: Alpha-1-antitrypsin, Neutrophil elastase, Chronic obstructive pulmonary disease

1. Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a global health problem affecting nearly 300 million people worldwide and killing 3 million individuals each year. COPD is the major cause of mortality will rise 7 to 8 million by year 2030 [1].

COPD is a disease of progressive, irreversible airflow limitation that is characterized by inflammatory response of lung to the noxious particles and gases [2]. In India, tobacco smoking is the major risk factor for the development of chronic obstructive lung disease [3]. However, currently there are no drugs available that can reduce progression of airflow obstruction in COPD patients [4,5].

Protease-anti-protease balance is required for normal function of the lung. Under normal conditions anti-proteases are present abundantly in lung tissue which balance the effect of neutrophil elastase and it provide anti-protease screen in the lungs. A shift of balance towards increased protease expression and activity can lead to increased inflammation and development of
chronic lung disease such as emphysema and chronic obstructive pulmonary disease [6].

Alpha-1-antitrypsin is also known as α1-protease inhibitor. Alpha-1-antitrypsin is the most abundant serine protease inhibitor in human lungs and it also present in plasma. Alpha-1-antitrypsin is a single polypeptide chain consists of 394 amino acids. Its molecular weight is 52000 Dalton [7,8]. Alpha-1-antitrypsin is mainly produced in liver. It is also synthesized in little amount in blood monocytes, macrophages, neutrophils and in pulmonary alveolar cells [9-11]. Alpha-1-antitrypsin has main function is to inhibit the activity of neutrophil elastase and to protect the lung against the deleterious effects of neutrophil elastase [12].

In healthy lungs, proteases are involved in cellular regeneration, cellular repair and tissue homeostasis [13]. In our study we studied role of protease (neutrophil elastase) in COPD patients. Neutrophil elastase is degrading enzyme released from neutrophils. Its main function is to destroy extracellular matrix, modifies airway epithelial cells and to disturbed respiratory host defense mechanisms. Neutrophil elastase acts as modulator of inflammation [14,15].

In previous study it has been reported that increased expression of neutrophil elastase leads to the development of emphysema [16,17]. Therefore, present study was aimed to see whether there is any correlation between levels of alpha-1-antitrypsin, neutrophil elastase with the lung function parameters in COPD patients. In addition to this, to see whether the neutrophil elastase /alpha-1-antitrypsin ratio can be use a marker for assessing the degree of severity of disease.

2. Materials and Methods

2.1.1. Selection of healthy controls: Control group consisted of 60 age and sex-matched healthy volunteers with no history of COPD, confirmed by spirometric tests performed during medical examination prior to the study.

2.1.2. Selection of study groups: COPD diagnosis for all patients included in the study was made by the evaluation of pulmonary function tests by using spirometer. Study subjects were aged in between of 40-75 yrs. Patients with all stages of COPD were included if they had a post-bronchodilator forced expiratory volume in one seconds (FEV₁)/force vital capacity (FVC) <70% after 400µg of inhaled salbutamol.

220 stable COPD patients were classified into four stages according to GOLD (Global Initiative for Obstructive Lung Disease) guidelines based on the post- bronchodilator values of FEV₁ % predicted with FEV₁/FVC % ratio <70% after performing lung function test, these are as follows:

a) Stage I COPD: (n=51, post-bronchodilator FEV₁ ≥ 80%, FEV₁/FVC % ratio <70%),

b) Stage II COPD: (n=57, post-bronchodilator FEV₁ ≥50% and <80%, FEV₁/FVC % ratio <70%),

c) Stage III COPD: (n=59, post-bronchodilator FEV₁ ≥ 30% and <50%, FEV₁/FVC % ratio < 70%),

d) Stage IV COPD: (n=53, post-bronchodilator FEV₁ < 30%, FEV₁/FVC % ratio <70%).

2.1.3. Inclusion Criteria

1. The patient was willing to participate in the study (i.e. patients written consent)

2. Smoking history of > 20 packs per year

3. Post bronchodilator increase in FEV₁ <12%

4. Patients were clinically stable (no exacerbation for 2 months) at the time of the evaluation.

5. Patients age >40 yrs were included in the study.

2.1.4. Exclusion Criteria for Controls and COPD patients groups: Patients who were suffering from or who were known to have tuberculosis, pneumonia, asthma, bronchiectasis, lung cancer, interstitial lung disease, respiratory failure, cardiac failure, diabetes mellitus, hepatic disease, renal disease and who had any recent surgical intervention and who were unable to performed lung function test were excluded from our COPD patients group.

Healthy individual with any past history of lung/respiratory disease or with abnormal lung function test were excluded from Control group.
Collection of Blood Samples: Under aseptic condition and with prior written consent of the subject, 4ml of blood was collected in plain bulb, allowed to clot for 1 hr. Serum was separated by centrifugation at 3000 rpm for 10 min. at room temperature, separated serum was aliquoted and stored at -80°C until the analysis and was used for the estimation of serum alpha-1-antitrypsin, neutrophil elastase and neutrophil elastase/alpha-1-antitrypsin ratio.

Pulmonary Function Test: Pulmonary Function test was done by using Spirometer.

Measurement of Forced Vital Capacity and Forced Expiratory Volume was done in First seconds.

The FEV1/FVC is calculated using the maximum FEV1 and FVC from the technically acceptable, though not from the same curves. Values of FEV1,% predicted, FVC and FEV1/FVC % ratio have been noted. The Data was obtained from the printer, attached to spirometer.

Estimation of Serum Neutrophil Elastase: Serum Neutrophil elastase was determined by ELISA method (Welldone Biotech., EIAab Science Co. Ltd, USA).

This method is based on the principle that micro titer plate provided in the kit has been pre-coated with antibody specific to neutrophil elastase.

Standards or samples are then added to the appropriate micro titer plate wells with biotin-conjugated polyclonal antibody preparation specific for Neutrophil elastase (NE) and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each well and incubated.

Then a TMB substrate solution is added to each well. Only those wells that contain neutrophil elastase biotin conjugated antibody and enzyme-conjugated avidin will exhibit a change in color.

The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at wavelength of 450nm. The concentration of Neutrophil elastase in the samples is then determined by compared the O.D. of the samples to the standard curve. It was expressed as pg/ml[18].

Estimation of alpha-1-antitrypsin: Enzyme linked immunosorbant assay (ELISA) test is used to determine human alpha-1-antitrypsin according to the “sandwich”-principle (Welldone Biotech., EIAab Science Co. Ltd, USA).

Alpha-1-antitrypsin in sample, standard and controls binds to antibodies, which are coated to the microtiterplate. After a washing step a peroxidase labelled detection antibody is added.

A second washing step is followed by the addition of the substrate which is converted to a colored product by peroxidase. The reaction is terminated by the addition of an acidic stop solution.

The optical densities are read at 450 nm (against the reference wavelength 620 nm) in a micro-titerplate reader.

The alpha-1-antitrypsin concentration can be calculated from the standard curve. The activity of alpha-1-antitrypsin is expressed in mg/dl [19].

Statistical Analysis: Statistical analysis was carried out by using Statistical Package for Social Sciences (SPSS 17 version) software. The data was expressed as Mean ± SD.

The statistical significance of the results among healthy controls and different stages of COPD patients was analyzed by using unpaired ‘t’ test.

One way analysis of variance (ANOVA) was used for to compare mean values in all groups followed by multiple comparisons between the groups was done by Tukey post hoc tests. P value of <0.05 was considered as statistical significant.

The strength of association between measured parameters among different stages of COPD patients with pulmonary function test parameters was analyzed by correlation coefficient analysis.

In addition to this, a receiver operating characteristic (ROC) curve was used to determine the optimal cut–off point for studied biochemical parameters as a classifier of COPD according to the highest sensitivity and specificity.

ROC curve was determined by using MedCalc Software.
3. Results

Table No.-1: Demographic Characteristics of patients with COPD and healthy controls involved in the study (the data expressed as mean ± SD)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Healthy Controls (n= 60)</th>
<th>COPD Stage I (n= 51)</th>
<th>COPD Stage II (n= 57)</th>
<th>COPD Stage III (n= 59)</th>
<th>COPD Stage IV (n= 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.93 ± 9.013</td>
<td>59.47 ± 9.30</td>
<td>62.91 ± 8.44</td>
<td>61.20 ± 7.45</td>
<td>61.73 ± 8.04</td>
</tr>
<tr>
<td>Smoking history</td>
<td>-----</td>
<td>52.1 ± 4.74</td>
<td>52.36 ± 7.25</td>
<td>53.56 ± 8.26</td>
<td>55.16 ± 8.97</td>
</tr>
<tr>
<td>BMI (weight in kg/ height in m²)</td>
<td>24.66±2.622</td>
<td>24.08±3.36</td>
<td>21.07 ± 3.278</td>
<td>19.97 ± 3.63</td>
<td>18.25 ± 2.90</td>
</tr>
<tr>
<td>FEV₁% Predicted</td>
<td>109.71 ± 14.90</td>
<td>86.17 ± 7.01</td>
<td>64.26 ± 7.57</td>
<td>41.16 ± 5.99</td>
<td>21.88 ± 4.18</td>
</tr>
<tr>
<td>FVC % predicted</td>
<td>110.16 ± 9.77</td>
<td>97.72 ± 12.42</td>
<td>66.91 ± 8.00</td>
<td>62.66 ± 7.45</td>
<td>48.28 ± 8.25</td>
</tr>
<tr>
<td>FEV₁/FVC % Ratio</td>
<td>103.58 ± 10.40</td>
<td>65.05 ± 4.6</td>
<td>62.40 ± 4.23</td>
<td>60.05 ± 4.93</td>
<td>57.51 ± 7.25</td>
</tr>
</tbody>
</table>

Values were expressed as Mean ± SD

a P= 0.155 statistically not significant as compare to healthy controls
b P= 0.0001 statistically extremely significant as compare to healthy controls
P= 0.001 statistically extremely significant as compare to stage I COPD
c P= 0.0001 statistically extremely significant as compare to stage II COPD
d P= 0.044 statistically significant as compare to stage III COPD
P= 0.0001 statistically extremely significant as compare to stage II COPD
e P= 0.003 statistically significant as compare to stage III COPD
f P= 0.003 statistically significant as compare to stage II COPD
g P= 0.001 statistically extremely significant as compare to stage III COPD
h P= 0.003 statistically significant as compare to stage II COPD
i P= 0.0001 statistically extremely significant as compare to stage II COPD
j P= 0.01 statistically significant as compare to stage III COPD

Table No.-2: Descriptive Statistic (mean ± SD) of Alpha-1-antitrypsin (mg/dl), Neutrophil elastase (pg/ml) and ratio of Neutrophil elastase / Alpha-1-antitrypsin ratio (mg/dl) in patients with different COPD stages and healthy controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy Controls Mean ± SD (n=60)</th>
<th>Stage I COPD Mean ± SD (n=51)</th>
<th>Stage II COPD Mean ± SD (n=57)</th>
<th>Stage III COPD Mean ± SD (n=59)</th>
<th>Stage IV COPD Mean ± SD (n=53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-1-antitrypsin (mg/dl)</td>
<td>161.6 ± 33.59</td>
<td>119.0 ± 24.9 a</td>
<td>105.4 ± 15.5 ab</td>
<td>73.5 ± 19.1 ad</td>
<td>54.7 ± 9.8 ad</td>
</tr>
<tr>
<td>Neutrophil Elastase (pg/ml)</td>
<td>287.0 ± 71.23</td>
<td>348.8 ± 92.8 a</td>
<td>392.7 ± 78.5 ae</td>
<td>435.5 ± 75.8 ae</td>
<td>478.1 ± 64.7 ae</td>
</tr>
<tr>
<td>Neutrophil elastase/ Alpha-1-antitrypsin ratio (mg/dl)</td>
<td>(1.86±0.64) X 10⁷</td>
<td>(3.11±1.2) X 10⁷ a</td>
<td>(4.30±1.04) X 10⁷ ab</td>
<td>(5.91±1.59) X 10⁷ ad</td>
<td>(7.14±2.70) X 10⁷ ad</td>
</tr>
</tbody>
</table>

P= 0.001 statistically significant when compared to healthy controls
b P= 0.016 statistically significant when compared to stage I
c P= 0.027 statistically significant when compared to stage I
d P= 0.0001 statistically significant when compared to stage I
Table No.-3: Correlation of protease (neutrophil Elastase), antiprotease (alpha-1-antitrypsin) with pulmonary function parameters in healthy controls and different Stages of COPD.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n=60)</th>
<th>Stage I (n=51)</th>
<th>Stage II (n=57)</th>
<th>Stage III (n=59)</th>
<th>Stage IV (n=53)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P value</td>
<td>r</td>
<td>P value</td>
<td>r</td>
</tr>
<tr>
<td>Neutrophil Elastase-FEV1 % predicted</td>
<td>+0.147</td>
<td>0.262</td>
<td>-0.760</td>
<td>0.000</td>
<td>-0.796</td>
</tr>
<tr>
<td>Alpha-1-antitrypsin-FEV1 % predicted</td>
<td>+0.179</td>
<td>0.171</td>
<td>+0.743</td>
<td>0.000</td>
<td>+0.789</td>
</tr>
<tr>
<td>Neutrophil Elastase-FVC % predicted</td>
<td>+0.083</td>
<td>0.528</td>
<td>-0.082</td>
<td>0.567</td>
<td>-0.392</td>
</tr>
<tr>
<td>Alpha-1-antitrypsin-FVC % predicted</td>
<td>+0.011</td>
<td>0.933</td>
<td>+0.037</td>
<td>0.796</td>
<td>+0.044</td>
</tr>
<tr>
<td>Neutrophil Elastase-FEV1/FVC % ratio</td>
<td>+0.090</td>
<td>0.494</td>
<td>-0.189</td>
<td>0.184</td>
<td>-0.412</td>
</tr>
<tr>
<td>Alpha-1-antitrypsin-FEV1/FVC % ratio</td>
<td>+0.047</td>
<td>0.721</td>
<td>+0.122</td>
<td>0.393</td>
<td>+0.269</td>
</tr>
<tr>
<td>NE - A1AT</td>
<td>-0.043</td>
<td>0.744</td>
<td>-0.350</td>
<td>0.01</td>
<td>-0.421</td>
</tr>
</tbody>
</table>

Fig.1. ROC curve analysis of NE/A1AT ratio in Stage I COPD patients

AUC=0.789,
Cut-off values of NE/A1AT for Stage I = 2.61 X 10^-7 mg/dl
Fig.2. ROC curve analysis of NE/A1AT ratio in Stage II COPD patients

AUC=0.813,
Cut-off values of NE/A1AT for
Stage II = 3.13 X 10^{-7} mg/dl

Fig.3. ROC curve analysis of NE/A1AT ratio in Stage III COPD patients

AUC=0.971,
Cut-off values of NE/A1AT for
Stage III = 4.35 X 10^{-7} mg/dl

Fig.4. ROC curve analysis of NE/A1AT ratio in Stage IV COPD patients

AUC=0.989,
Cut-off values of NE/A1AT for
Stage IV = 4.97 X 10^{-7} mg/dl
Table No.1 shows that irrespective of the sex in healthy volunteers the normal FEV$_1$% predicted and FEV$_1$/FVC % ratio was observed, which decreases with the advancement of the stage.

Table no. 2 shows mean ± S.D values of alpha-1-antitrypsin, neutrophil elastase and neutrophil elastase/alpha-1-antitrypsin ratio in healthy controls and different stages of COPD patients. In our study, we observed significantly decrease levels of alpha-1-antitrypsin (A1AT) from stage I to stage IV COPD patients as compared to the levels in healthy controls. When we did intergroup comparison of mean values of serum A1AT level of stage I vs. stage II, III and stage IV we found significantly decrease levels of A1AT in stage II, III and IV COPD (Table no.2).

As shown in table no.3 we found significant positive correlation of A1AT with FEV1% predicted in stage I, II, III and in stage IV COPD patients. In addition to this, we obtained positive correlation between A1AT with FVC% only in stage III and stage IV COPD. We observed positive correlation between FEV$_1$/FVC % ratio with A1AT in stage II, III, IV COPD patients (table no.3). As shown in table no.3 we found strong negative correlation between serum neutrophil elastase with alpha-1-antitrypsin (r = -0.350, P = 0.01; r = -0.421, P = 0.0003; r = -0.667, P < 0.001; r = -0.948, P = 0.0001) respectively in stage I, II, III and IV of COPD.

Neutrophil elastase is a degrading enzyme. Mean ± SD values of serum neutrophil elastase as shown in table no.2. In our study we observed increased levels of neutrophil elastase from stage I to stage IV of COPD as compared to the healthy controls. When intergroup comparison was done we found statistically significant increase activity of serum neutrophil elastase in all stages of COPD according to the severity of the disease (table no.2). As shown in table no. 3 we found significant negative correlation between neutrophil elastase with FEV1% predicted in stage I, II, III and IV COPD. We obtained inverse correlation between FVC% predicted and FEV1/FVC % ratio with neutrophil elastase only in stage II, III and in stage IV COPD (table no.3). To test the ability of serum neutrophil elastase/alpha-1-antitrypsin ratio as biomarker the cut off value of neutrophil elastase/alpha-1-antitrypsin ratio was determined by ROC curve analysis. The cut-off point value of NE/A1AT ratio for stage I 2.61 X 10$^{-7}$ mg/dl (AUC=0.789, SE= 0.03, 95% CI= 0.69 to 0.85, P=0.0001), 3.13 X 10$^{-7}$ mg/dl for stage II (AUC=0.813, SE=0.02, 95%CI=0.74 to 0.87, P<0.0001), 4.35 X 10$^{-7}$ mg/dl for stage III (AUC= 0.971, SE=0.01, 95%CI= 0.91 to 0.99, P<0.0001), 4.97 X 10$^{-7}$ mg/dl for stage IV (AUC=0.898, SE=0.01, 95%CI= 0.94 to 0.99, P<0.0001). At this cut-off levels of Neutrophil elastase/alpha-1-antitrypsin ratio, we achieved a sensitivity of 74% (95%CI=60.5 to 85.2) and specificity of 60.7 % (95%CI=46.5 to 72.4) respectively for stage I, a sensitivity of 80.70% (95%CI=68.0 to 90.0) and specificity of 88.3% (95% CI= 77.4 to 95.2) for stage II, a sensitivity of 98.30% (95%CI=90.0 to 100.0) and specificity of 100% (95% CI= 94.0 to 100.0) for stage III, a sensitivity of 100% (95%CI=93.3 to 100) and specificity of 100% (95% CI= 94.0 to 100.0) for stage IV (Fig. no. 1, 2, 3 and 4)

4.Discussion

Free radicals present in the tobacco smoke that directly penetrate into the respiratory tract system, then reach the lung alveoli, generate reactive oxygen species and other oxidants/ free radicals which is the main factor for the development of COPD[20]. Free radical not only damage lipids, proteins, DNA but also modulates some processes such as increased production of mucus, impaired cilia function, loss of elasticity of the alveoli of lungs, increased airway obstruction that leads to the development of COPD [21]. In our study we found significantly decreased levels of FEV$_1$% predicted, FVC% predicted, FEV$_1$/FVC % ratio in all stages of COPD patients (table no.1). Daphne CR et al observed similar observation in their study [22]. This decline in lung function parameters might be due to the structural changes in the airways and alveoli of lungs of COPD patients which include inflammation in lung tissues, airway remodeling, mucus hypersecretion, bronchospasm, increased airway resistance and loss of elastic recoil resulting in progressive decreased expiratory airflow. This decreased airflow obstruction causes generation of reactive oxygen species and free radicals.

The lungs destroy most of the toxins, particles and infectious agents because of its large surface area before any organ does. Tobacco smoking, inhalation of air pollutant and bacterial infection result in lung irritation and migration of neutrophils.
and macrophages to these area of stress [23,24]. Neutrophil elastase is a powerful protease released from the neutrophils, it destroys the extracellular matrix, modifies airway epithelial cells and it disturbed respiratory host defense mechanisms [25]. An increased level of serum neutrophil elastase has been reported in disease of cystic fibrosis, acute respiratory distress syndrome (ARDS) and lung cancer patients [26-28]. Information regarding serum levels of neutrophil elastase in COPD remains scant. To the best of our knowledge, we are first to report serum levels of neutrophil elastase in different stages of COPD patients. In present study, we observed increased levels of neutrophil elastase from stage I to stage IV of COPD as compared to the levels in healthy controls.

When intergroup comparison was done we found statistically significant increase in serum neutrophil elastase in all stages of COPD according to the severity of the disease (Table no.2). Similar to our study, Borzi et al has reported elevated levels of serum neutrophil elastase in patients with idiopathic pulmonary fibrosis (IPF) [29]. The observed increased activity of neutrophil elastase in COPD patients could be due to overproduction of oxidants at the site of lung inflammation which oxidizes the methionine residue at the active site of alpha-1-antitrypsin therefore its inhibitory capacity on neutrophil elastase was lost, it cannot binds to the neutrophil elastase resulting more neutrophil elastase to release in the lungs and then into pulmonary circulation [30,31]. As a regards neutrophil elastase there were significant negative correlation between their levels with FEV$_1$/FVC % predicted, FEV$_1$/FVC % ratio in stages of COPD patients.

Protease–Antiprotease balance is essential for the normal lung function. Normally, the lung is adequately protected against this neutrophil elastase by alpha-1-antitrypsin. A1AT is a protein that rapidly binds to these proteases, thereby it irreversibly inhibits their proteolytic activity. Alpha-1-antitrypsin is an important antiprotease. A1AT is synthesized in hepatocytes, neutrophils, macrophages, blood monocytes and in pulmonary alveolar cells. Various investigators have studied the protease- antiprotease imbalance in the development of respiratory conditions like bronchiectasis, bronchial asthma and in emphysema [32-34]. To the best of our knowledge, we are first to report serum levels of A1AT in different stages of COPD patients. In current study, we observed significantly decrease levels of A1AT from stage I to stage IV COPD patients as compared to the levels in healthy controls. When we did intergroup comparison of mean values of serum A1AT level of stage I vs. stage II, III and stage IV we found significantly decrease levels of A1AT in stage II, III and IV COPD (Table no. 2). These possible decrease levels of A1AT in COPD patients could be due to oxidants modified the structure of A1AT. This results in conformational change occurred in the structure of A1AT, resulting alpha-1-antitrypsin limits the passage of liver to blood circulation and then to the lungs [35].

Therefore the availability of A1AT in the lung and in blood circulation may be less in stages of COPD patients. Similar to our study, Gaillard et al has reported markedly reduced plasma elastase inhibitory capacity in asthmatic patients as compared to non-asthmatic patients [36]. In contrast to our study, Jadhav BS et al (2013) reported increased levels of alpha-1-antitrypsin in stages of COPD patients [5]. In our study we obtained positive correlation of A1AT with FEV$_1$/FVC % ratio with A1AT in stage II, III, IV COPD patients. A1AT correlated positively with FVC% only in stage III and in stage IV COPD. We observed direct correlation between FEV$_1$/FVC % ratio with A1AT in stage II, III, IV COPD patients (Table no.3). In contrast to our finding, Rai RR et al [6] have reported negative correlation between A1AT and FEV$_1$ % predicted in COPD patients. In correlation study we found strong negative correlation between serum NE with alpha-1-antitrypsin (r = -0.350, P = 0.01; r = -0.421, P=0.0003; r = -0.667, P < 0.001; r = -0.948, P = 0.0001, table no.3) respectively in stage I, II, III and IV of COPD.

The normal function of the lungs depends on the protease–antiprotease balance. In physiological conditions activity of neutrophil elastase is inhibited by α-1-antitrypsin. In oxidative stress condition α-1-antitrypsin becomes functionally inactive due to oxidation of methionine residue on active site of enzyme. Therefore inhibitory action of α-1-antitrypsin on neutrophil elastase is lost resulting more NE released in the lungs. Neutrophil elastase enzymes break down elastin, collagen,
proteoglycan and laminin components of extracellular matrix of lung tissues [25], therefore increased fibrosis of lung tissues and that leads to development of emphysema and COPD. In our study we observed increased levels of neutrophil elastase/α-1-antitrypsin ratio from stage I to IV of COPD as compared to healthy controls (P=0.001).

When intergroup comparison was done of serum neutrophil elastase/α-1-antitrypsin ratio of stage I vs. stage II, stage III and stage IV of COPD we found significant increased in protease/antiprotease ratio in stage II, III, IV COPD as compared to stage I (P=0.012, P=0.001, P=0.001) respectively (Table no.2). From these findings, we suggest that this neutrophil elastase/α-1-antitrypsin ratio can tell us the severity of the chronic obstructive pulmonary disease in terms of increased fibrosis of the lung tissue.

To prove the efficacy of Neutrophil elastase/Alpha-1-antitrypsin ratio as a biomarker we did ROC curve analysis which revealed the cut-off values of neutrophil elastase/alpha-1-antitrypsin ratio 2.61 X10^{-7} mg/dl for stage I (AUC=0.789, SE= 0.03, 95% CI= 0.69 to 0.85, P<0.0001), 3.13 X 10^{-7} mg/dl for stage II (AUC=0.813, SE=0.02, 95%CI=0.74 to 0.87, P<0.0001), 4.35 X10^{-7} mg/dl for stage III (AUC= 0.971, SE=0.01, 95%CI= 0.91 to 0.99, P<0.0001), 4.97 X 10^{-7} mg/dl for stage IV (AUC=0.989, SE=0.01, 95%CI= 0.94 to 0.99, P<0.0001).

At this cut-off levels of Neutrophil elastase/alpha-1-antitrypsin ratio, we achieved a sensitivity of 74% (95%CI=60.5 to 85.2) and specificity of 60.7% (95%CI=46.5 to 72.4) respectively for stage I, a sensitivity of 80.70% (95%CI=68.0 to 90.0) and specificity of 88.3% (95% CI= 77.4 to 95.2) for stage II, a sensitivity of 98.30% (95%CI=90.0 to 100.0) and specificity of 100% (95% CI= 94.0 to 100.0) for stage III, a sensitivity of 100% (95%CI=93.3 to 100) and specificity of 100% (95% CI= 94.0 to 100.0) for stage IV (Figure no 1,2,3 and 4)).

From our findings we can say that determination of serum levels of Neutrophil elastase/Alpha-1-antitrypsin ratio can be used as good classifier for discriminating the degree of severity in COPD patients, when patients is not able to performed spirometry.

5.Conclusion

From this result we conclude that neutrophil elastase and alpha-1-antitrypsin these circulating markers are candidate predictors for rapid decline of lung function in COPD patients. Neutrophil elastase/α-1-antitrypsin ratio can tell us the severity of the chronic obstructive pulmonary disease in terms of increased fibrosis of the lung.

Though the magnitude of neutrophil elastase/alpha-1-antitrypsin ratio is minute, still it can be a good marker of pulmonary function in COPD patients (proved by ROC curve analysis).

Ethical Approval: The study protocol was examined and authorized by the Medical Research and Ethics Committee of the B.J. Govt. Medical College and Sassoon General Hospital in accordance with the ethical standards laid down in 1964 Declaration of Helsinki on biomedical research on human subjects. We have obtained necessary Institutional ethical approval [Ref.No. BJMC/IEC/Pharmac/D1210133-35]

Strength and Limitations of the Study

Strength: We are the first here to report the correlation between serum level of Neutrophil elastase, alpha-1-antitrypsin with the markers of airflow obstruction (FEV_{1},% predicted) in COPD patients.

Limitations: The present study was carried out with a relatively small number of subjects. It would be better to perform this study with greater number of subject in order to determine the difference between the analysis of biochemical and lung function parameters of COPD patients and healthy control groups more clearly.

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