Plasma Antiprotease Status in Different Respiratory Disorders

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Citation

R Rai, M Phadke. *Plasma Antiprotease Status in Different Respiratory Disorders*. The Internet Journal of Pulmonary Medicine. 2006 Volume 7 Number 1.

Abstract

In the present study we estimated plasma concentration and activity of antiproteases in control group and in the study groups of patients with chronic obstructive pulmonary disease (COPD), emphysema, bronchiectasis and bronchial asthma. α_1-proteinase inhibitor (α_1-PI) concentration was significantly increased in all study groups as compared to control group. In patients with emphysema, bronchiectasis, and bronchial asthma. α_2-macroglobulin (α_2-M) concentration was significantly increased as compared to control group. But in patients with COPD, α_2-M concentration was comparable to that of control group. Antitryptic and antielastase activity in condition of COPD was found to increase significantly. But in the other three study groups antitryptic activity was found to decrease. Antielastase activity was not found to vary significantly in bronchiectasis, and was found to decrease significantly in emphysema and bronchial asthma. Significant correlation between antiproteases concentration, antiproteases activity and pulmonary function tests was found only in some study groups.

INTRODUCTION

The lung encounters most of the toxins, particles and infectious agents in our environment before any other organ does[1]. Cigarette smoking, excessive inhalation of polluted air, and respiratory infections result in lung irritation and the migration of phagocytic cells to these areas of stress. A major component released from human neutrophil granules during the process of phagocytosis is a proteinase referred to as human neutrophil elastase. This enzyme has been shown to catalyze the degradation of all of the major connective tissue components – including elastin, collagen, and proteoglycan and it is believed to be primarily responsible for the destruction of lung alveoli associated with the development of pulmonary emphysema[2]. Normally, the lung is adequately protected against proteases by antiproteases, proteins that rapidly bind to proteases, thereby irreversibly inhibiting their proteolytic activity, α_1-proteinase inhibitor (α_1-PI), secretory leukoprotease inhibitor (SLPI), and α_2-macroglobulin (α_2-M) are antiproteases central to the pulmonary antiproteolytic defenses[3].

α_1-PI is an important plasma proteinase inhibitor (52 kDa) and is synthesized by hepatocytes and macrophages[4]. α_1-PI acts against trypsin, chymotrypsin, plasmin and possibly thrombin, but the inhibition of greatest clinical significance is against neutrophil elastase and collagenase. α_2-M is a large glycoprotein (720 kDa) of plasma and is synthesized by a variety of cell types, including monocytes, hepatocytes and astrocytes[4]. α_2-M inactivates endopeptidases from all four classes (seryl, cysteinyl, aspartyl, metallo). α_2-M is an effective inhibitor of human neutrophil elastase , however it is less efficient than α_1-PI[5].

Proteases-antiproteases balance is essential for the normal lung function. Various investigators have studied the protease-antiprotease balance in the development of different respiratory conditions like COPD, emphysema, bronchiectasis and bronchial asthma[6,7,8]. COPD is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases[9]. Emphysema is a condition of the lung characterized by abnormal permanent enlargement of the air spaces distal to the terminal bronchiole, accompanied by destruction of their walls[10]. Bronchiectasis is the chronic abnormal dilatation and distortion of bronchi caused by destruction of the elastic and muscular component of the bronchial wall[10]. Asthma is an inflammatory disease of the airways in which the mucous membrane and layers of the bronchi become thickened and the mucous glands enlarge, reducing airflow in the lower respiratory tract[11].

Pulmonary function tests are widely used in the evaluation and management of patients with known or suspected disorders of respiration. The volumes of air moving in and out of the lungs and remaining in them are of great importance. Forced vital capacity (FVC) is the maximum volume of air which can be breathed out as forcefully and rapidly as possible following a maximum inspiration. Clinical significance of FVC is to distinguish between restrictive and obstructive lung disorders. FEV_1 is the volume of air expired in 1st second of exhalation[12].

The aim of the present work was to study and estimate plasma concentration and activity of antiproteases in control group and in the study groups of patients with COPD, emphysema, bronchiectasis and bronchial asthma, and to find out correlation between these estimations and pulmonary functions of patients, if any.

MATERIALS AND METHODS

All chemicals were of the highest purity available and used as received. α_1-PI concentrations and α_2-M concentrations were estimated by using commercial kits and were purchased from Spinreact, Spain. Plasma total protein was determined by using commercial kit and was purchased from Quali Test, Rashmi Diagnostic Pvt. Ltd. Porcine pancreatic elastase (EC 3.4.21.36) and N-Succinyl-Ala-Ala-Ala p-nitroanilide were purchased from Sigma Chemical Co., St. Louis, MO. Bovine trypsin was purchased from, S.D. fine-chem Pvt. Ltd. Boisar. α-N-benzoyl-DL-arginine-p-nitroanilide (BAPNA) was purchased from Fluka. Sodium chloride (NaCl), Tris (hydroxymethyl) Aminomethane (tris buffer) were purchased from Qualigens Fine Chemicals Mumbai. All the other chemicals were of analytical reagent grade.

The patients included in the study were from respiratory medicine OPD of Lokmanya Tilak Municipal Medical College and General Hospital Sion, Mumbai.

The study group included 111 patients. Further patients were subgrouped depending upon the diagnosis as : COPD = 24, Emphysema = 20, Bronchiectasis = 32 and Bronchial asthma = 35. The control group included 50 healthy, non-smokers who had no history of lung disease and had normal pulmonary function tests.

Morning venous blood samples were collected for the study of various parameters and taken in EDTA containers. The plasma collected by centrifugation for 10 min. was either tested immediately or frozen and stored at – 25°C.

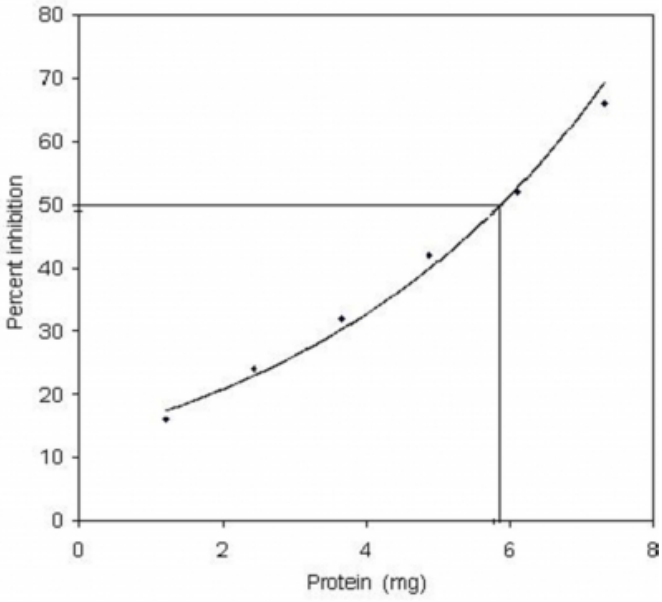
The various parameters estimated were, Plasma α_1-PI concentration, Plasma α_2-M concentration, Antitryptic activity and Antielastase activity.

The determination of α_1-PI concentration and α_2-M concentration was based on the reaction between α_1-PI and α_2-M as antigen and the specific antiserum as antibody. This reaction forms an insoluble complex producing a turbidity which is measured spectrophotometrically at 340 nm. The results were evaluated using a reference curve prepared with the aid of calibrator dilutions.

Antitryptic activity was determined by incubating trypsin and its substrate BAPNA with different volumes of plasma and without plasma and estimating the mg of plasma protein required to inhibit the enzyme to the extent of 50% at optimum pH and optimum temperature condition.The antitryptic inhibitors of plasma inhibit the hydrolysis of α-N-benzoyl-DL-arginine-p-nitroanilide (BAPNA) by trypsin in tris buffer, pH 8.0. The reaction is stopped by adding acetic acid (30% v/v) and the absorbance of p-nitroaniline, liberated after hydrolysis is read at 410 nm. The percent inhibition of trypsin was calculated by comparing with positive control (without plasma) which is taken as 100% activity. The mg of protein required for 50% inhibition was found from the plot of the protein concentration (mg) against percent inhibition[12,13] (Figure 1).

Figure 1

Figure 1 : Antitryptic activity of plasma.



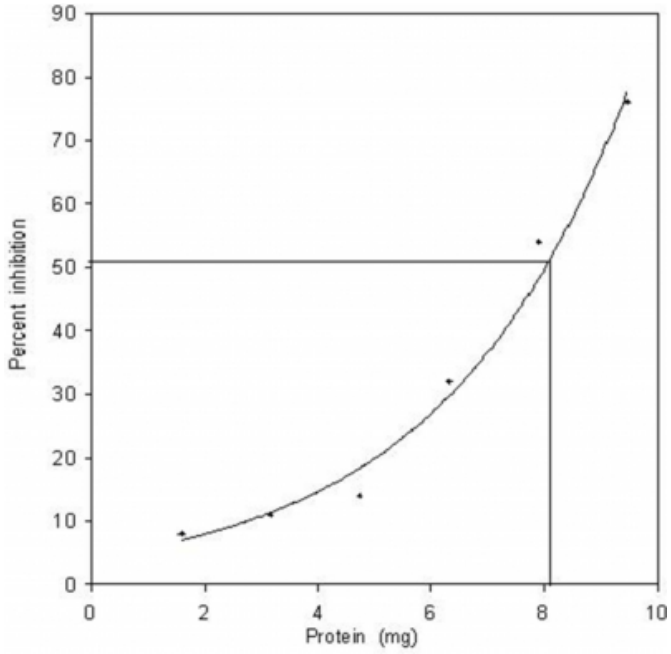
Activity of α_1-PI is measured by estimating the mg of plasma protein required to inhibit the enzyme (trypsin-20µg) to the

extent of 50% at optimum pH & optimum temperature conditions.

Antielastase activity was determined by incubating enzyme elastase and its substrate N-Succinyl -Ala-Ala-Ala-p-nitroanilide with different volumes of plasma and without plasma and estimating the mg of plasma protein required to inhibit the enzyme to the extent of 50% at optimum pH and optimum temperature condition.The antielastase inhibitors of plasma inhibit the hydrolysis of N-Succinyl -Ala-Ala-Ala-p-nitroanilide by elastase in tris buffer, pH 8.0. The reaction is stopped by adding acetic acid, and the absorbance of p-nitroaniline liberated after hydrolysis is read at 410 nm. The percent inhibition of elastase was calculated by comparing with positive control (without inhibitor) which is taken as 100% activity. The mg of protein required for 50% inhibition was found from the plot of the protein concentration (mg) against percent inhibition[12] (Figure 2).

Figure 2

Figure 2 : Antielastase activity of plasma.



Activity of α_1-PI is measured by estimating the mg of plasma protein required to inhibit the enzyme (elastase-1.5µg) to the extent of 50% at optimum pH and optimum temperature conditions.

Pulmonary function tests were performed in all patients and in control subjects. Forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) were measured with Ocean Win Sipro Software. FEV_1 and FVC were expressed as a percentage of the predicted values for age, sex, and height.

STATISTICAL ANALYSIS

Statistical analysis was carried out using unpaired ‘t' test. P value less than 0.05 ($P<0.05$) was considered as significant.

RESULT & DISCUSSION

Proteases and antiproteases balance is essential for the normal lung function. In the healthy lung, the proteolytic activity of proteases is balanced by presence of antiproteases locally. Under certain conditions, when there is an increased protease burden which is not compensated by antiprotease activity, lung injury follows. Also, when antiprotease defense is decreased, lung parenchymal changes may result[14]. A number of diseases involving the lung, such as COPD, emphysema, bronchiectasis and bronchial asthma have been associated with a disturbance of these balances[6,7,8].

Human plasma is a rich source of different proteinase inhibitors (antiproteases) whose function is to control the proteolysis by affecting the proteinase activity. α_1-PI and α_2-M are the two important proteinase inhibitors present in plasma. α_1-PI is the chief proteinase inhibitor present in highest amount inhibiting the serine proteinases elastase and trypsin[4]. α_1-PI, an “acute-phase-reactant” may increase during pregnancy, during bacterial infections, after thyphoid vaccine, in the event of severe burns, or in the presence of malignant tumors and decrease in pulmonary emphysema, cirrhosis of the liver and noninfectious hepatitis in the neonate[15]. α_2-M is an effective inhibitor of both human neutrophil elastase and cathepsin G[5]. α_2-M is not an “acute-phase-reactant”. Increased α_2-M levels have been reported in pulmonary disorders, diabetes mellitus and in patients with liver disease[16].

In the present study, we estimated the plasma concentration of α_1-PI and α_2-M and also total antitryptic and total antielastase activity of plasma. Table 1 shows, in patients with COPD, emphysema, bronchiectasis and bronchial asthma, α_1-PI and α_2-M concentration is increased as compared with control. This might be the body's response to deal with increased proteolytic activity so that balance is maintained.

Figure 3

Table 1: Comparison of α-PI concentration and α-M concentration between study and control groups.

Study Groups		Control n=50	COPD n=24	Emphysema n=20	Bronchiectasis n=32	Bronchial Asthma n=35
α_1-PI mg/dl	Range	130-210	130-288	132-223	146-240	133-260
	Median	165.0	196.5	196.5	191.5	184.0
	± SD	23.36	38.43	24.55	25.35	32.48
	p Value		p<0.001	p<0.001	p<0.001	p<0.001
α_2-M mg/dl	Range	133-270	146-330	163-313	181-373	177-400
	Median	205.0	223.5	246.5	234.5	250.0
	± SD	32.28	50.76	44.15	54.87	52.84
	p Value		NS	p<0.001	p<0.01	p<0.001

Values expressed as median ± SD

NS= Not significant

Similar to our study Denchev and colleagues (1977) estimated the α_1-PI in patients with bronchial asthma with different forms and severity of the disease. α_1-PI was found to be elevated in all patient groups. The elevated α_1-PI concentration may be explained as a response of allergic process and secondary bacterial inflammatory reactions8.

The functional capacity of the α_1-PI is markedly susceptible to oxidative inactivation. This inactivation appears to be due to the oxidation of a critical methionine residue at the elastase inhibitory site of α_1-PI. Reduced levels of elastase inhibitory activity in the lung can result from the partial oxidation of α_1-PI through inhalation of oxidants present in tobacco smoke, ozone, or industrial gases, as well as from oxidants released by lung macrophages and other phagocytic cells[17].

The activity of α_1-PI and α_2-M as judged by inhibition of trypsin and elastase on their respective substrates, in vitro, was found to vary in different conditions (Table 2).

Figure 4

Table 2 : Comparison of total Antitryptic and Antielastase activity of plasma between study and control group.

Study Groups		Control n=50	COPD n=24	Emphysema n=20	Bronchiectasis n=32	Bronchial Asthma n=35
Total Antitryptic activity**	Range	5.4-10.5	4.4-9.5	6.25-10.6	6.35-11.3	5.7-16.9
	Median	7.475	6.00	9.125	8.3	7.9
	± SD	1.3	1.43	1.40	1.53	2.18
	p Value		p<0.001	p<0.001	p<0.001	NS
Total Antielastase Activity**	Range	4.8-11.55	5.0-15.0	6.1-12.05	5.45-13.05	5.15-12.9
	Median	7.8	6.75	9.6	7.9	8.6
	± SD	1.53	2.38	1.84	1.70	1.75
	p Value		p<0.001	p<0.001	NS	p<0.001

Values expressed as median ± SD

NS= Not significant

** : mg of plasma protein required to inhibit the enzyme (trypsin - 20µg, elastase - 1.5µg) to the extent of 50% at optimum pH & optimum temperature conditions.

In the condition of COPD, both antitryptic and antielastase activity of plasma increased significantly ($p<0.001$) as compared to control, which may be because of increased concentrations of both α_1-PI and α_2-M. But in the other groups antitryptic activity decreased inspite of increased concentration of the inhibitors. Antielastase activity was not found to vary significantly in conditions of bronchiectasis and was found to decrease significantly ($p<0.001$) in emphysema and bronchial asthma. Similar to our study Gaillard and colleagues showed that plasma elastase inhibitory capacity was markedly reduced inspite of increased values of α_1-PI in asthmatic patients as compared with nonasthmatic individuals[18].

Literature shows smoking is associated with the loss of lung elasticity in α_1-antitrypsin deficiency[19] and also to the decrease in lung function. Pulmonary function tests are useful in patients with chronic airway obstruction to confirm the obstructive abnormality, to quantify the severity of the defect, to assess the reversibility of the airflow obstruction in response to therapy[20], and to monitor the course of the disease.

In the present study we estimated pulmonary function parameters of patients and control group (Table 3).

Figure 5

Table 3 : Pulmonary Function Tests in different study groups.

Study Groups		Control n=50	COPD n=24	Emphy-sema n=20	Bronchi-ectasis n=32	Bronchial Asthma n=35
FEV_1	Range	91.0-101.0	28.0-80.0	11.0-65.0	15.0-108.0	21.0-93.0
	Median	96.00	48.50	29.50	67.00	59.00
	± SD	2.22	16.37	17.58	21.90	17.29
	p Value		p<0.001	p<0.001	p<0.001	p<0.001
FVC	Range	88.0-99.0	42.0-95.0	28.0-85.0	13.0-106.0	29.0-94.0
	Median	97.00	61.00	47.00	77.50	70.00
	± SD	2.83	16.16	16.90	20.96	16.77
	P Value		p<0.001	p<0.001	p<0.001	p<0.001

And we also investigated whether antiproteases concentration and antiproteases activity are related to lung function tests in all groups (Table 4).

Table 4 : Correlation coefficient (R) between different parameters and PFT (% Predicted) in different study groups.

Pulmonary function tests of the study group shows significant reduction in FEV_1 and FVC as compared to control group. This clearly indicates obstruction of airways in all the conditions under study i.e. COPD, emphysema, bronchiectasis and bronchial asthma. The cause and mechanism of development of these pathologies may be different. Correlation coefficient values (Table 4) showed that significant correlation between aniproteases concentration, antiproteases activity and pulmonary function tests was found only between some parameters in different study groups.

Thus uniformity is seen in all the disorders under study that there is blockage of airways and there is shift in proteases-antiproteases balance towards proteases as reflected by the measurement of antitryptic and antielastase activity. Whether this shift is cause or effect of the disorder remains to be studied.

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