Correlation of Alpha-1 Antitrypsin and Smoking in Chronic Obstructive Lung Disease: An Observational Study

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Research Article

Abstract: **Setting-** The present study was carried out in the Department of Physiology, Biochemistry & Medicine Govt. Medical College, Aurangabad, Maharashtra, India. **Objective-** To correlate serum AAT levels in smoker and non smoker COPD patients with controls. **Design-** An observational study carried out in 314 subjects in the age group 40-70 years from medicine department of our college divided into three groups. **Group 1-** Normal healthy individuals without history of smoking (controls).**Group 2-** Patients with COPD for 5 years or more without history of smoking in present or past. **Group 3-** Patients with COPD for 5 years or more with history of smoking of at least 10 pack years. Pulmonary function tests were used for COPD diagnosis as per GOLD criteria and Serum AAT levels done by turbidimetric method. Analysis was done by ANOVA, Pearson's correlation coefficient and Tukey's test. **Results-** The results indicate that serum AAT levels were decreased in non smoker COPD patients (187.7 ± 55.42) & smoker COPD patients (133.37 ±19.36) when compared to controls (251.5 ± 47.73). Smoker COPD patients show lowest values of serum AAT. **Conclusion-** So serum AAT levels can act as predictor for future development of COPD in smokers & in non smokers.

Keywords: COPD, FEV1, GOLD criteria, Serum AAT, Healthy controls.

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a major cause of chronic morbidity and mortality throughout the world. [1] The chronic airflow limitation is caused by a mixture of small airway disease (obstructive bronchiolitis) and parenchymal destruction (emphysema). It causes destruction of the lung parenchyma leading to the loss of alveolar attachments to the small airways and decrease in lung elastic recoil. These changes diminish the ability of airways to remain open during expiration.

Cigarette smoking is the most commonly encountered risk factor for COPD.[2] COPD mortality is predicted by age of starting to smoke, total pack-years smoked, and current smoking status. [3] Even though 90% COPD patients are related to history of smoking, paradoxically only 20% of smokers develop COPD. [4] Thus role of smoking in pathogenesis of COPD remains debatable. Environmental pollution and genetic factors are other risk factors contributing to COPD pathogenesis. [2]

Severe deficiency of alpha 1 antitrypsin (AAT) was identified as an important risk factor causing COPD by Laurell and Eriksson. Very low levels of AAT cause diffuse panacinar pulmonary emphysema, beginning in young adult life. [5] Malerba M et al [6], Senn O et al [7] and Sandford AJ et al [8] suggest that the increased risk of development of COPD is seen in all AAT genotypes.

Risk ratios for COPD in AAT deficiency range from 1.5 -12 fold, depending on whether the AAT deficiency is present in heterozygous or homozygous combinations. Among normal individuals only 1 in 100 has a deficient allele. However, among individuals with COPD, up to 10% have a deficient allele. [9]

AAT is an inhibitor of neutrophil elastase (NE), a destructive proteolytic enzyme stored in neutrophils. AAT protects extracellular structures from attack by neutrophil elastase released by activated or disintegrating neutrophils. [10] The protease-antiprotease theory of emphysema predicts that an imbalance between proteases, such as neutrophil elastase, and inhibitors, such as AAT causes progressive destruction of the alveoli, which culminates in emphysema. [11]

Cigarette smoking accelerates the onset of disease by increasing the number of neutrophils (and neutrophil elastase) in the alveolus and inactivating the remaining small amounts of antiprotease. [5]

The references suggest that AAT deficient people develop COPD but there are very few studies that signify values of AAT in patients of COPD and if there is any relation of COPD as a disease and

smoking as a risk factor for lowering the values of serum AAT levels. So, the present study was carried out to investigate the relationship between serum AAT levels and development & progression of COPD in smokers and non smokers.

Material and Method:

The present study was an observational descriptive study carried out in the Departments of Physiology, Biochemistry & Medicine at Govt. Medical College, Aurangabad, Maharashtra. Study protocol was approved by Institutional Ethical Committee, GMC Aurangabad. Patients were recruited for six months (Jan 2009 – June 2009) from the outpatient department and admitted patients of COPD. Age group selected was 45 to 75 years. The Diagnosis of COPD was done by PFT's using GOLD criteria for diagnosis of COPD updated 2008. [2] Age & sex matched controls free from COPD were taken as control. Informed written consent was obtained from each subject.

314 subjects selected for study were divided into three groups

Group 1- Normal healthy individuals without history of smoking (control). (n=108)

Group2- Patients suffering from COPD for 5 years or more without history of smoking in present or past. (n=102)

Group3- Patients suffering from COPD for 5 years or more with history of smoking of at least 10 pack years. One ''pack year'' is 20 cigarettes/80 beedis smoked /day for one year. (4 beedis are equivalent to 1 cigarette). [12]

Exclusion criteria:

- Subjects suffering from other diseases like diabetes, hypertension, or cardiac disorders.
- Subjects having cancer or have had cancer in the 5 years prior to study entry or having undergone lung surgery (e.g. lung reduction, lung transplant).
- Subjects suffering from other respiratory disorders, or disorders e.g.: asthma, lung cancer, sarcoidosis, tuberculosis, lung fibrosis.
- Terminally ill patients of COPD.
- Serious, uncontrolled disease (including serious psychological disorders) likely to interfere with the study or impact on subject safety.

Pulmonary function tests: PFTs were done in all study groups. Two consecutive sputum samples were tested before PFT to rule out tuberculosis. PFT 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, FEV_1 and FEV1/FVC.

Measurement of serum alpha1 antitrypsin levels: Serum AAT was estimated by test kit of SPINREACT Company. Basic principle of the method is anti α_1-antitrypsin antibodies when mixed with samples containing AAT form insoluble complexes. These complexes cause an absorbance change, dependent upon the AAT concentration of the patient's sample that can be quantified by comparison from a calibrator of known AAT concentration. This method of AAT estimation in human serum of plasma is quantitative turbidimetric method. The method includes Tris buffer 20 mmol/L as diluent & anti human α_1-antitrypsin antibodies derived from goat serum.

Serum AAT levels were estimated in 314 persons from three study groups. Venous blood of 1 ml was taken as sample in EDTA bulb. Fresh serum or plasma was separated by centrifuging the sample. Assay is performed on XL640 Auto analyzer (Trans Asia Company.) Blood was taken from smoker subjects at least 12 hours after the last puff of smoke to avoid immediate effect of smoking.

Standardization:

Calibrator having known value i.e. 372mg/ml of α 1 AT activity was serially diluted & the readings are taken on XL 640 Trans Asia auto analyzer.

CONC	0	37.2	93	186	279	372
absorbance	0.06	0.45	0.74	0.89	0.9	0.91

The normal serum levels of AAT are 20-53 µmol/l (150-350mg/dl) About 95% of patients with severe AAT deficiency serum levels decrease about 15% of normal (5-6 µmol/l (30-40mg/dl). [13]

The data was analysed by following tests using Graph Pad Prism 5 statistical software:

ANOVA was used for comparison of demographic data and data of serum AAT values in control, non smoker COPD & smoker COPD groups. Inter group comparison was done with Tukey's multiple comparison test.

Pearson's correlation coefficient test was used for correlation of serum AAT levels and predicted FEV_1 % in smoker and non smoker COPD patients.

Result

For the selection of control group, 350 relatives of patients coming to medicine department fulfilling the selection criteria were matched for age, sex and BMI with the cases& screened by their PFT results. 108 matched subjects were selected as controls having FEV1/FVC > 70%.

All the patients of 45- 65 years age coming in medicine OPD with more than 5 years history of COPD were screened for exclusion criteria. PFT's were done before and after bronchodilation. The patients with FEV1/FVC < 70% and not improving more than 12% or 200ml post bronchodilation were taken as cases. The patients were divided into smoker (n=104) and non smoker group (n=102).

The results in control group show that serum AAT levels were 251.5 ± 47.73mg/dl with confidence interval of 242.39 to 260.60. FEV1% predicted was 90.16 ± 8.18 and FEV1/FVC was 84.09 ± 5.42.

In non smoker COPD patients serum AAT levels were 187.7 ± 55.42 mg/dl with confidence interval of 176.81 to 198.58. FEV1% predicted was 42.7 ± 16.59 and FEV1/FVC was 53.27± 10.63.

In smoker COPD patients serum AAT levels were 133.37 ± 19.36 mg/dl with confidence interval of 129.61 to 137.13. FEV1% predicted was 48.76 ± 16.59 and FEV1/FVC was 43.83 ± 8.43.

On analysis by using ANOVA the differences in AAT levels between the three groups was statistically highly significant with 'p' value of <0.0001. Pearson's correlation test show significant positive correlation between serum AAT levels and FEV1 % predicted in both smoker (coefficient=0.4097) and non smoker (coefficient=0.352) COPD groups.

Table 1: Table showing the demographic data of the three study groups

Parameter	Control (n=108)	Non-smoker COPD patients (n=102)	Smoker COPD patients (n=104)	P-value (One way ANOVA)
Age (years) (Mean ± SD)	53.87 ± 8.85	54.47 ± 8.23	55.10 ± 7.95	0.8176 (NS)
Weight (Kg) (Mean ± SD)	60.53 ± 8.94	58.73 ± 10.01	58.50 ± 12.91	0.7427 (NS)
Height (m) (Mean ± SD)	1.62 ± 0.07	1.58 ± 0.06	1.59 ± 0.07	0.750 (NS)
BMI (Mean ± SD)	23.14 ± 3.50	23.48 ± 4.37	22.98 ± 4.24	0.9661 (NS)

* Statistically significant

Table 2: Table showing comparison of Serum AAT levels in four groups

Serum AAT levels (mg/ml)	Predicted FEV_1%	Pearson's correlation coefficient
133.37 ± 19.36	48.76 ± 16.59	0.4097* (between 0 to 1)

* Statistically significant

Table 3: Table showing correlation of serum AAT levels and predicted FEV_1 % in non smoker COPD patients.

Serum AAT levels (mg/ml)	Predicted FEV_1%	Pearson's correlation coefficient
187.7 ± 55.42	42.7 ± 16.59	0.352* (between 0 to 1)

* Statistically significant

Table 4: Table showing correlation of serum AAT levels and predicted FEV_1 % in smoker COPD patients.

Parameter	Controls (Mean ± SD)	COPD non- smokers (Mean ± SD)	COPD smokers (Mean ± SD)	One way ANOVA (p value)
Serum AAT (mg/dl)	251.5 ± 47.73	187.7 ± 55.42	133.37 ±19.36	<0.0001***

*** Statistically highly significant

Discussion:

In present study serum AAT values were decreased in COPD in smoker group & non smoker group as compared to control group.

The pathogenesis of COPD in the patients is that low serum AAT favors the spontaneous formation of AAT loop sheet polymers within the lung. This conformational transition inactivates AAT as a proteinase inhibitor, thereby further reducing the already depleted levels of AAT that are available to protect the alveoli. The polymers are themselves chemotactic for human neutrophils in vitro. They may evade the defense mechanisms of the lung by adhering to the interstitium causing early onset COPD changes. [14]

The values were more significantly decreased in smokers indicating an important role of smoking in pathogenesis of COPD. Smoking allows unopposed action of proteolytic enzymes specially accelerated at low pH. [11]

F. Ogushi has shown that cigarette smoking puts the individual at risk for the development of emphysema as AAT molecules in smokers take longer time to inhibit neutrophil burden than in non smokers. [15]

Charles Mittman et.al. in 1971 showed that patients with intermediate levels of protease inhibitor became ill as age advances and following greater cigarette exposure. [16]

However, result of the study of Somayajulu G.L et.al is contradictory to present study and other studies. They revealed no significant difference in AAT levels between the two groups of smokers and non smokers. However in this study significantly lower serum AAT levels were seen in 5 out of 21 smokers suggesting association of smoking with AAT levels. [17]

This descriptive study has shown that correlation exists between serum AAT levels and COPD as well as smoking. So, it leads to question as to whether AAT levels can be taken as a parameter to determine the progress of COPD & therefore can be used as an important tool in management of COPD. Also, studies need to be done to ascertain if COPD & smoking affect AAT levels and whether correction of AAT levels can benefit the COPD patients to halt or reverse the progress of disease.

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