

Association of Alpha-1-Antitrypsin with Asthma

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ABSTRACT

Introduction: Asthma is a chronic inflammatory disease of the airways that affects people of all ages. Alpha-1-antitrypsin (or alpha-1-proteinase inhibitor) is the most abundant circulating proteinase inhibitor. Alpha-1-antitrypsin deficiency has a high morbidity and mortality. Alpha-1-antitrypsin deficiency may present with symptoms that are similar to those of asthma. Aim of the present study was to determine the level of alpha-1-antitrypsin activity in case of asthma patients to assess the possibility of misdiagnosis which leads to increase morbidity.

Material and Methods: The present study included the patients diagnosed with asthma in the department of T.B. and Respiratory Diseases in a span of one year. Blood samples of Patients between age group of 31 to 65 years were collected and analyzed for alpha-1-antitrypsin activity for the present study. The method that was adopted in the present study of serum alpha-1-antitrypsin activity was by measuring the inhibition of trypsin by serum.

Results: The serum alpha-1-antitrypsin level was found to be 2.37 ± 0.13 mg/ml among the control group, which lies in the normal range. The range of serum alpha-1-antitrypsin activity of the control group is 2 to 2.56 mg/ml. The serum alpha-1-antitrypsin level was found to be 2.26 ± 0.36 mg/ml among the sixty asthma patients. Amongst them six patients had alpha-1-antitrypsin less than 2 mg/ml. The range of serum alpha-1-antitrypsin activity of the asthmatic patients was 0.94 to 2.67 mg/ml.

Conclusion: Early diagnosis by screening of all asthmatic patients as well as family members of confirmed alpha-1-antitrypsin deficiency cases is needed to decrease incidence of morbidity and mortality in affected individuals.

Keywords: Proteinase Inhibitor; Serpin Superfamily; Neutrophil Elastase; Serine, Antiprotease; Wheezing, Antielastase, Chronic Obstructive Pulmonary Disease

and undertreated in many parts of the world.⁵ Asthma has long been recognized as a condition with a strong genetic component.⁶ May 3 is an annual event organized by the Global Initiative for Asthma as World Asthma Day, to improve asthma awareness and care around the world.

Alpha-1-antitrypsin deficiency (A1ATD) is an autosomal co-dominantly inherited disease which mainly affects the lungs and liver.⁷ It can be homozygous or heterozygous in character. Homozygotes have alpha-1-antitrypsin below 35% of the normal concentration while the level in heterozygotes is 40-80% of normal.⁸ In normal and healthy individuals, alpha-1-antitrypsin, a 52-Kd serine antiprotease, protects the lungs from enzymatic digestion by neutrophil elastase that helps fight bacteria and clean up dead lung tissue. This if allowed uninhibited on the lung parenchyma gives rise to destruction of the alveoli.⁹ Enumerable studies have been done on A1ATD since it was first recognized by Laurell and Eriksson in 1963.¹⁰ To date, more than 100 genetic variants of the AAT gene (*SERPINA1*) have been identified. While the principal site of synthesis is the liver parenchymal cell,¹¹ alpha-1-antitrypsin synthesis also occurs in lung-derived epithelial cells mononuclear phagocytes, neutrophils, intestinal epithelium, kidney parenchyma and several other sites.

Although the etiology and disease mechanisms of asthma and A1ATD are distinct, patients with A1ATD commonly first present with asthma-like symptoms¹² such as shortness of breath, wheezing, chronic cough and recurring chest colds and initially receive asthma treatment.¹³ A1ATD is often not diagnosed in patients with asthma because of the similarity in symptoms. The average Alpha patient experiences symptoms for more than 8 years and sees at least 3 doctors before being correctly diagnosed with Alpha-1 antitrypsin deficiency.¹⁴ This delay results in destruction of lung tissue prior to diagnosis.

Asthma patients are divided into severity groups according to the International Classification of Asthma.¹⁵ In alpha-1-antitrypsin deficiency, the presence of asthma, increases the risk for development of Chronic Obstructive Pulmonary Disease.¹⁶ Inherited homozygous alpha-1-antitrypsin

INTRODUCTION

Global Strategy for Asthma Management and Prevention (2015 update) describe asthma as a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation.¹ These episodes are usually associated with airflow obstruction. The obstruction is often reversible with treatment. American Thoracic Society guidelines specifically included asthma with an irreversible component within Chronic Obstructive Pulmonary Disease.² Currently more than 300 million individuals are suffering from asthma worldwide, about 10% of whom live in India.^{3,4} Both studies in India and International surveys have demonstrated that asthma is still underdiagnosed

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deficiency is the strongest single risk factor. Cigarette smoking also contributes in the loss of some of the normal antielastase protection of the lower respiratory tract, making smokers more vulnerable to destructive lung diseases.¹⁷ Smokers were also included in the study. In the present study smoking status was defined as follows: "A smoker is a person who has smoked at least 20 packs of cigarettes or at least one cigarette per day for at least one year in a life time while an ex-smoker is a person who has abstained from smoking for at least three months, and a never smoker is a person who is not a smoker or an ex-smoker".¹⁸ Although asthma is a heterogeneous disease that may present at any age, it often becomes apparent during childhood and occurs more frequently in youth. In contrast, the clinical manifestations of A1ATD are rarely seen before 25 years of age and more often occur as hepatic illness when presenting in childhood.¹⁹ In adults, a high proportion of individuals with alpha-1-antitrypsin deficiency having symptoms suggestive of asthma have been reported.

There are 3 possible interactions between alpha-1-antitrypsin deficiency and asthma. First, a common disease such as asthma can occur coincidentally with alpha-1-antitrypsin deficiency. Second, it is obvious that airflow obstruction with wheezing and productive cough occurring in a person with alpha-1-antitrypsin deficiency could readily be misdiagnosed as asthma. Third, the carrier state could predispose to the development of asthma. Once asthma develops in patients with alpha-1-antitrypsin deficiency, the antiprotease and anti-inflammatory effectiveness of any alpha-1-antitrypsin present in the airways may be functionally reduced.²⁰ Aim of the present study was to determine the level of alpha-1-antitrypsin activity in case of asthma patients to assess the possibility of misdiagnosis which leads to increase morbidity.

MATERIAL AND METHODS

The present study was planned and conducted in the Department of Biochemistry and Department of T.B. and Respiratory Diseases at Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh (India). Sixty serum samples were collected from normal healthy male and female adults. These sera served as controls. This sample size had matching characteristics in respect of age and sex as that of study group (Fig: 1). Study group also comprised sixty samples (Fig. 2) from patients attending J. N. Medical College, Hospital, Aligarh, with clinical manifestations pointing to asthma. Detailed history and thorough clinical examination of patients was done and investigations such as haemogram, X-Ray chest, PA View and Lateral View (if needed), sputum for AFB (if needed), culture sensitivity, eosinophil count, routine urine examination and Lung Function Test (if needed). Patients were educated about the study's procedure. A written informed consent was obtained from them for the present study. Approval was obtained from the Medical College before beginning the study. Samples of blood were collected from patients and serum separated from the sample and subjected to measurement of serum trypsin inhibitory activity according to the procedure of Waheed and

Salahuddin (1975)²¹ also described elsewhere by the author.²² In the present study most of asthma cases presented dyspnea and wheezing as the primary symptoms.

Blood samples were collected separately and were planned for serum alpha-1-antitrypsin activity measurement. The serum trypsin inhibitor activity of all samples was determined by measuring the inhibition of trypsin by the sera according to the method of Waheed and Salahuddin (1975).²¹ The substrate that has been used for trypsin is N Alpha-Benzoyl-DL-Arginine P-Nitroanilide (BAPNA). Trypsin was allowed to react with BAPNA resulting in the formation of P-nitroanilide which has intense yellow colour. After the reaction of trypsin with BAPNA, the absorbance of the solution was measured colorimetrically at 410 nm. The intensity of colour was used for checking the activity of trypsin in the presence and absence of serum.

STATISTICAL ANALYSIS

All the results were analyzed by SPSS software. Demographic details of the patients were also recorded. Student t test, Pearson correlation coefficient, and regression equation analysis were used for the assessment of level of significance. P-value of less than 0.05 was considered as significant.

RESULTS

Demographic details including the percentage of males and females in different age groups, smokers and severity of diseases of the healthy subjects (control) and the asthmatics in the present study are emphasized in Table 1. Mean age of the healthy subjects included in the present study is higher than that of asthmatic cases. Of the total 60 healthy subjects, those in 31-40 year age group were 17 (28.33%), among which 9 (15%) are males and 8 (13.33%) are females. In 41-50 year age group healthy subjects were 19 (31.67%), amongst which 9 (15%) are males and 10 (16.67%) are females. In 51-60 year age group 13 (21.67%) subjects were there. Amongst them, 7 (11.67%) were males and rest 6 (10%) were females. Rest of the 11 healthy controls in more than 60 years age group 5 (8.33%) were males while rest 6 (10%) were females.

The 60 asthmatic patients were lying in different age groups. In 31-40 years age group 23 (38.33%) patients were there. Amongst them 18 (30%) were males and 5 (8.3%) were females. In 41-50 years age group 20 (33.33%) patients were there. Amongst them 12 (20%) were males and 8 (13.33%) were females. In 51-60 year age group there were 10 (16.67%) patients. Of these 8 (13.33%) were males and 2 (3.33%) females. In more than 60 year age group there were 7 (11.67%) patients. Of these 5 (8.33%) were males and 2 (3.33%) were females. Patients were divided into three asthma severity groups according to the signs, symptoms and investigations. Among the 60 asthmatic cases, 50% (30) patients were lying in moderate severity group, while 14 (23.33%) were mild, and 16 (26.67%) were severe cases (Fig. 1).

The range of serum alpha-1-antitrypsin activity of the

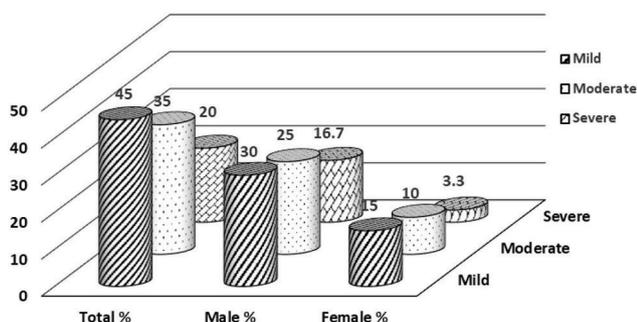


Figure-1: Severity of study cases (Asthmatic)

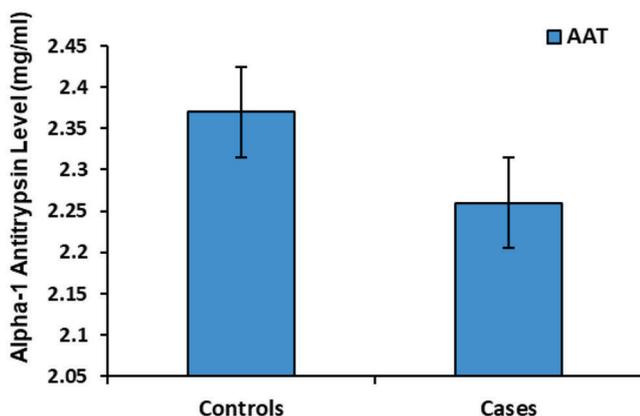


Figure-2: Alpha-1 antitrypsin level in control and asthmatic cases

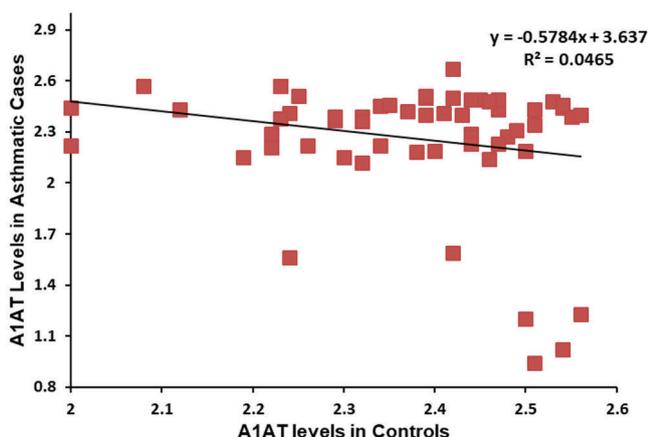


Figure-3: Negative correlation graph

	Controls	Asthma cases
Sample Size	60	60
Mean of Alpha-1 antitrypsin level (mg/ml ± SD)	2.37±0.13	2.26 ± 0.36
Mean Age (years)	48.35	45.37
SEM	0.017	0.04
Smokers	16%	35%
Standard Deviation (SD); Standard Error Mean (SEM)		

Table-1: Demographic details of the patients

control group is 2 to 2.56 mg/ml. The mean value of serum alpha-1-antitrypsin activity came out to be 2.37±0.13 mg/ml (Mean ± S.D) in the controls (Fig. 2). The range of serum alpha-1-antitrypsin activity of the patients was 0.94 to 2.67 mg/ml. The mean value of serum alpha-1-antitrypsin

activity came out to be 2.26±0.36 mg/ml (Mean ± S.D) in the asthmatic patients (Fig. 2). Of the studied sixty asthmatic cases, six had alpha-1-antitrypsin level less than 2 mg/ml. Amongst these 4 were males and 2 were females. These males and female were between the age group of 31-50 years. They were mainly moderate to severely asthmatic. *P* value is 0.0270 which is significant. Student's *t*-test value is 2.2397, indicating the averages of the two sample groups are significantly different. while (degree of freedom) *df* = 118. Standard error of difference = 0.051.

Inverse correlation between controls and asthmatic cases with alpha-1 antitrypsin was established (Fig. 3), as evident from the values (Pearson correlation coefficient, *r* is -0.237). In case of regression analysis, displayed a decline and this relationship was also supported by negative regression ($y = -0.5782x + 3.637$, $R^2 = 0.046$) where R^2 is coefficient of determination.

Among the 60 asthmatic cases, 21 (35%) were smokers. Among them 20 (33.33%) were males and 1 (1.67%) was female. Among the 35% smokers eighteen i.e. 30% had normal levels of alpha-1-antitrypsin activity while 3 (5%) were alpha-1-antitrypsin deficient.

DISCUSSION

The serum alpha-1-anti-trypsin level of the control group lies in the normal range. Of the studied sixty cases of asthma fifty-four had normal alpha-1-antitrypsin level. This finding is in agreement of our as well as of other researchers.²² While A1AD is an underdiagnosed disease,²³ the relationship between bronchial asthma and A1AD is debatable. The mechanism by which AAT deficiency could predispose to asthma is not well known.²⁴ The frequency of reported asthma in alpha-1-antitrypsin deficiency has been highly variable.⁸

The presence of inflammation is necessary for development of clinically significant asthma. Inflammation added to a deficiency of antiprotease inhibitor deteriorates bronchial hyperreactivity. Alpha-1-antitrypsin deficiency could be important in the pathogenesis of inflammatory processes and in the clinical manifestations characteristic of patients with intrinsic asthma.²⁵

Previously it was believed that alpha-1-antitrypsin is rarely found in dark and Asian populations. But recently publications on the worldwide racial and ethnic distribution of AAT deficiency, suggest AAT deficiency in other major racial groups worldwide also including Arabs and Jews in the Middle East and Southeast Asians. The new data will affect the diagnosis of AAT deficiency.²⁶ In the present study 6 cases had alpha-1-antitrypsin level below 2mg/ml in asthmatic patients. Further, all the 6 cases of decrease level were in the age group of 31-50 years. The incidence of alpha-1-antitrypsin deficiency was found to be higher in males than females i.e. 5 males and 1 female. Eden et al., (1997) suggested that a lack of alpha-1-antitrypsin in airways intensifies the tendency to develop asthma.¹² They noted that asthma is more common in alpha-1-antitrypsin deficiency group than in individuals without alpha-1-antitrypsin

deficiency.

Of the 6 cases of alpha-1-antitrypsin level below 2mg/ml in asthmatic patients, 50% were smokers also and exhibited coexistence with decrease in alpha-1-antitrypsin activity. This indicates association between cigarette smokers and alpha-1-antitrypsin deficiency. In asthmatics who smokes, cigarettes may exacerbate pulmonary symptoms while obscuring the component that another underlying disease exists. Asthma cases which show lack of improvement after receiving the usual therapy and such cases should be evaluated for AATD screening.¹⁹ Findings of earlier researchers also indicate that though alpha-1-antitrypsin deficiency seems to appear later in nonsmokers but once initiated it progresses at the same rate in smokers as well as nonsmokers with severe alpha-1-antitrypsin deficiency.²⁷ With smoking on the increase in women, it is possible that women may catch up with men in terms of absolute numbers.

The diagnosis and treatment of asthma is a sensitive issue.³ There have been a limited number of investigations on this issue in India in the past. The current supplement on asthma guidelines, a joint initiative by the Indian Chest Society (ICS) and the National College of Chest Physicians (NCCP), pulls together a locally relevant set of guidelines for general and pulmonary physicians of this country.⁴

CONCLUSION

Alpha-1-antitrypsin deficiency is a good example of the impact of environmental factors on the phenotype of a genetic disease. While awareness of the problem is the first essential, these are all the more necessitated as the worldwide adoption of cigarette smoking proceeds at an alarming rate.

According to the World Health Organisation (WHO) all individuals with COPD, emphysema or asthma or with a family history of the disorder should be screened once for A1ATD using a quantitative test.²⁸ ATS (American Thoracic Society) / ERS (European Respiratory Society) and WHO provide recommendations for genetic testing in order to enhance the detection of severe A1ATD patients.^{28,29} Owing to overlapping clinical features, A1ATD is often overlooked in the differential diagnosis of asthma and can be misdiagnosed as asthma. The possible relationship between partial deficiencies of this enzyme and bronchial asthma remains controversial.

Current asthma guidelines recommend a control-based approach to management that involves assessment of impairment and risk followed by implementation of treatment strategies individualized according to the patient's needs and preferences. Alpha-1 is easily diagnosed with simple blood tests. Although genetic testing was not done in our study but Genetic testing, coupled with confirmation of the patient's phenotype, is the optimal method of diagnosing A1ATD. Awareness to A1ATD should be done by educational campaigns and increase in availability of low cost/ free test kits. The diagnostic delay is significant, and efforts should be directed to increase early A1ATD detection. Correct and early diagnosis is important for the therapy which is different for each of them.

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