

## Original Article

## Assessment of Alpha-1 Antitrypsin Deficiency in Patients with Severe Chronic Obstructive Pulmonary Disease

Nezarali Moulaei<sup>1</sup> M.D., Hossein Ali Khazaei<sup>2\*</sup> Ph.D., Vida Pejman<sup>3</sup> M.D.

<sup>1</sup>Internal Medicine Department, Research Centre for Tropical and Infectious Diseases, Zahedan University of Medical Sciences, Zahedan, Iran.

<sup>2</sup>Immunology and Hematology Department, Clinical Immunology Research Center, Zahedan University of Medical Sciences, Zahedan, Iran.

<sup>3</sup>Zahedan University of Medical Sciences, Zahedan, Iran.

### ABSTRACT

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**Background and Aims:** Chronic obstructive pulmonary disease (COPD) is a kind of pulmonary diseases characterized by chronic obstruction of lung that is in the form of a diffuse narrowing of airways resulting in air flow resistance. Alpha-1 antitrypsin (AAT) deficiency is genetically relatively common risk factor in patients with COPD throughout the world and the exact cause of its prevalence is unknown. We therefore performed a study to determine the frequency of AAT deficiency in patients with severe COPD compared to the healthy controls.

**Materials and Methods:** In this cross-sectional case control study, AAT serum level in 60 patients with severe COPD for whom the history and spirometry test with FEV1<50% had been confirmed based on gold criteria as well as 60 healthy controls, were tested using commercial kit and nephelometry method.

**Results:** The lowest serum levels of AAT measured in patients was <0.349 g/l and the highest was 3.099 g/l. These were obtained in healthy subjects as 1.180 g/l and 4.195 g/l respectively. Out of 60 patients, 4 (6.7%) had partial deficiency of AAT (AAT<1 g/l) and 6 (10%) had definite shortage of AAT (AAT<0.5 g/l). In healthy subjects, we did not find any definite and relative lack of AAT. The comparison of results obtained from these two groups indicated a significant difference between frequency of AAT (P=0.001).

**Conclusions:** Our findings revealed the frequency of AAT deficiency, as a factor involved in COPD disease, to be 10% and can be the reason for the high prevalence and severity of COPD in Zahedan city.

## Introduction

Chronic obstructive pulmonary disease (COPD) is one of the most common respiratory disorders in adults with lung being obstructive and progressive which is irreversible and is accompanied by diffuse narrowing of the airways associated with increased resistance to airflow [1]. The cause of obstruction is inflammation and increased mucus production of respiratory tract leading to reduction of the air flow to bronchi and bronchioles [2]. The disease symptoms are shortness of breath, cough, excessive sputum production, wheezing, increased levels of carbon dioxide in the blood, decreased oxygen, and intolerance to physical activity (fatigue) [3]. Systemic manifestations of disease include secondary polycythemia, anxiety, skeletal muscle dysfunction, increased frequency and severity of attacks usually accompanied by decreasing quality of life which gradually become progressive at first [4]. This disease is the fourth cause of death and the main cause of chronic disability in the world which has affected more than 10 million people in America. In addition, the importance of COPD in public health is rising all over the world. According to the estimates, COPD, probably as the sixth cause of mortality in the world will take the third rank in 2020. About 25 percent of population over age 40 are infected with COPD [2]. There are many other problems in the way to determine the true prevalence of COPD that is due to different methods of sampling, differences in response to treatment,

spirometry devices and incorrect use of bronchodilators drugs [5].

Kind of COPD includes emphysema that has an anatomic definition and is characterized by destruction and enlargement of the pulmonary alveolar, chronic bronchitis with cough and sputum, and small airways tightened. This disease also reported to be associated with diseases such as asthma, cardiac vascular disease, anemia and depression [2, 3]. Although asthma and COPD both arise from chronic inflammatory response but the type of cells involved in this process and inflammatory mediators involved in this process and inflammatory mediators involved in it are different as a result of symptoms of these two diseases and response to treatment in them, but in extreme cases asthma and COPD course of these two diseases are similar. In addition some features of COPD and asthma are similar and asthma symptoms of the smokers will arise similar to COPD [6, 7]. However, the research conducted in 29 countries from 1990 to 2004 has revealed that COPD in +40-years-old smokers and former smokers is more prevalent than in non-smokers and in – 40 years old, its prevalence is higher in men than women. Despite the problems in COPD-related statistics, it remains one of the major causes of death in most countries of the world [8-10]. Smoking has been identified to be an important risk factor for COPD which causes change in health policy in many aspects. Although much work has been done around smoking and its

association with COPD, this is not the only risk factor as many researches show that COPD incidence is identified in non- smokers as well [10].

In 1964, the Advisory Committee of the General Association of America reported that smoking is a risk factor for the mortality of COPD patients being due to chronic bronchitis and emphysema. Although gender differences has been reduced in rates of smoking in the past 50 years, the prevalence of this disease in women is increasing [2, 10]. Even the people who are exposed to smoking (passive smokers) can be at the risk of COPD in terms of the overall burden of respiratory tract [11, 12]. On the other hand, age of starting smoking, number of packs, years of smoking, and smoking status are now criteria for determining the probability and severity of symptoms of COPD. Of course not all smokers clearly suffer from COPD and this pronounces the role of other factors especially heredity [13, 14]. Although changes in lifestyle and urbanization had increased cases and deaths, smoking is still the most important factor for COPD that affects 10 to 15 percent of the smokers; other environmental and occupational factors such as exposure to dust are also effective [15, 2]. Genetic factors such as violations of the enzymes like alpha-1 antitrypsin (AAT) are found to be a cause of this disease but in a small percent of patients, there is a clear lack of it [3].

ATT is an inhibitor serum serine proteases involved in blood coagulation and complement system. AAT has got its name for its ability to inhibit trypsin enzymes, collagenase and

trypsin holozoic cells in the laboratory setting, but its main role is to inhibit neutrophil elastase and create emphysema [16-18]. ATT is kind of acute phase proteins in human serum with six-day half life and is synthesized in the liver and its serum concentrations is 1.3 g/L and its level changes in liver, kidney and lung diseases. Serum level of 50-80 mg/dl is defined for the deficiency of AAT [16, 19]. ATT deficiency disease is the potential genetic risk factor in outbreak of COPD disease and deficiency of this protein was for the first time introduced in 1062 with different gene mutation. The most common type of it being from S and Z located on 14q 31-32.3 human chromosome mainly defect PIZZ, lead to bringing its concentration in serum and tissues to lower than 50 µg/dl. Ninety five percent of patients are the possessors of PIZZ alleles even if they are with heterozygotes with severe defects [20, 21]. Deficiency of this enzyme causes 1 to 2 percent of cases of COPD and leads to outbreak of early emphysema; usually smoking exacerbates its incidence rate and in some cases becomes asthma [22]. Its prevalence in Iran was reported by Nadi and colleagues in Hamedan but its shortage was not observed among the studied patients [23]. The highest global prevalence of this deficiency in 2008 has been reported in Madyar-Portugal [24]. Deficiency of this enzyme with other diseases, including asthma, vacuities, aneurysms, ulcerative colitis, positive ANCA, glomerular disease, pany colitis or inflammation of subcutaneous tissue and finally through the ejection changes it to the inhibitor of the coagulation factors which

also leads to bleedings that threatens the patient's lives [25, 26]. The exact statistics for prevalence of this disease in different regions of the world as well as in this country and the city of Zahedan is not available but there are some reports of link of this deficiency to COPD. The first report of prevalence of AAT deficiency in COPD and other respiratory diseases was reported in 1977 by Kulpati [27]. In another study done by Bowne in 1996, 2.7% of patients with obstructive pulmonary disease who have died between 1979 and 1991 were identified as patients with shortage of AAT [28]. Later on Limber and colleagues in 1986 announced that 8% of COPD patients compared to 2.9% of healthy controls had variants of transported genotype (heterozygous) deficiency of AAT (PIZ) and 1.9% of COPD patients compared to 0.04 healthy control had homozygous PIZZ variants and have severe shortage of this enzyme [29]. As its lack with the disorientation of the enzyme in the lung causes various disabilities, death and economically leads to enormous costs of treatment and care, this study was designed to determine the amount of this protein in the serum of patients with severe COPD compared with healthy individuals in order to better understand if the deficiencies are observed, appropriate control programs such as using antibiotics, doing further investigation through the study of genotype of this protein by molecular techniques with polymerase chain reaction are needed to better determine the lack of this enzyme and finally control environmental agents.

## Materials and Methods

In the present cross-sectional case control study, 60 severe COPD patients as well as 60 healthy controls were studied. Diagnosis of severe COPD disease in 60 patients was confirmed through spirometry review with  $FEV_1 < 50\%$  (before and after using bronchodilator) and biography. The exact serum levels of AAT in patients and normal individuals were measured with nephelometry method and commercial kit purchased from commercial label Bender Med kit (Bioscience-UK). The Ethics Committee of Zahedan University of Medical Science, Zahedan, Iran approved this protocol.

## Statistical Analysis

Statistical analysis was performed with SPSS version 17 (SPSS Inc, Chicago, IL, USA). Comparison of the frequency of AAT in two groups was reported as a percentage of this enzyme in total population. A difference in the amount of AAT in two groups was evaluated using T test. Significant differences between frequency of AAT in two patients and control group ( $P < 0.05$ ) confirmed the associations according to the Mann-Whitney method.

## Results

The results of this study revealed that 19 patients (31.7%) were male and 41 (68.3%) were female. The mean age of these patients was 59.76 years and their age range was 40 to 78 years. The minimum serum levels of AAT measured in these patients were  $< 0.349$  g/l

and the maximum amount were 3.0991 g/l. According to the previous observations and researches, the amount of AAT for a definite shortage was 0.5 g/l and for the relative shortage was 1 g/l which was determined through nephelometry method [23]. So according to the mentioned criteria, out of 60 examined patients, 6.7% had a relative shortage, 10% had definite shortage and 83.3% of patients had normal AAT. The minimum amount of AAT in healthy people was 1.18 g/l and maximum was 4.195 g/l. According to the previous studies, the amount of AAT for a define shortage was 0.5 g/l and for relative shortage was smaller than 1g/l which was determined through nephelometry method. In healthy persons, no cases of defined and relative shortage of AAT were found. According to the results, there is a significant difference of the frequency of AAT between

the two patients and the control groups (P=0.001) (see Table 1). The results of this study showed that In patients with definite shortage of ATT enzyme, 3 patients were male, (50%) and 3 female (50%). 2 (33.3%) had occupational pollution and 6 (%100) suffered from a history of smoking and shisha. On the other hand, further demographic analysis of these patients revealed that out of 60 studied patients, 29(48.3%) had smoking and shisha history and out of this number, 17 patients (58.62%) were female and 12 (41.38%) male. 29 patients (48.3%) had high risk of jobs-created COPD. 57 patients (95%) were suffering from cough and sputum. In 31 patients (51.7%), some degrees of heart failure was observed. 36 patients (60%) were from urban and 24 (40%) from rural districts.

**Table 1.** Comparison of AAT frequency in severe COPD patients and normal individuals

ATT Group	Relative deficiency		Definite deficiency		Normal	
	Number	%	Number	%	Number	%
Patients	4	6.7	6	10	50	<b>83.3</b>
Healthy controls	0	0	0	0	60	<b>100</b>

## Discussion

In this study, the association between AAT and severe COPD was investigated. The study population was composed of 60 COPD patients with a mean age of 59.76 years who had been hospitalized in Ali-Ebne-Abitaleb hospital in Zahedan as well as 60 healthy controls. Data gathering was performed using questionnaire form and spirometry result test. The association between AAT and respiratory diseases has been of interest to many

investigators. From the beginning, this association was first introduced with severe early onset of emphysema in five cases of AAT and was originally published in 1963 [17]. Later AAT screening for asthma was also reported elsewhere by Siri in 2013 [26]. This concept has changed the knowledge about the pathophysiology of emphysema including the role of inflammation and, in particular, the biological role of proteolytic enzymes. Thus this provides facilities for scientists to better

find out the impact of this protein on the basis of biochemistry, genetics, cell biology, and disease concepts outside the lung as well as the study of COPD in general.

Little is known about the ATT screening for severe COPD disease on the bases of serum concentration of this enzyme. As far as our knowledge concerns, the genetic linkages leading to deficiency of ATT associated with the disease has been clarified and the pathophysiologic processes, clinical variation in phenotype and the role of genetic modifiers have been recognized [1, 17]. In a meta-analysis of Hersh et al. (2004) conducted on patients suffering from COPD, the results showed a proven genetic risk factor for COPD by relation of severe ATT to homozygosity for the protease inhibitor Z allele [30]. Another genetic study done by Molloy et al. in 2014 revealed that severe AAT especially PIZZ homozygosity has been correlated with increased risk of airflow obstruction and emphysema, they did not show certainly the risk of COPD in PIMZ heterozygotes [1]. Regarding the serum concentration of this enzyme in COPD diseases, this has been investigated through our study and our results confirmed definite shortage of this enzyme in COPD patients compared to the healthy controls. Our result also showed that 10% of severe COPD hospitalized patients had definite shortage of this enzyme.

According to the studies performed in Asia, Arab race, Eastern race, Turkish and Far East, the deficiency of this enzyme has been reported to be very rare. Furthermore, according to the study that has been

accomplished in 2006 in Hamadan based on the investigation of this enzyme regarding COPD patients, their results confirm finding no shortage of this enzyme. On the other hand, based on reports from European races, the prevalence of ATT deficiency in COPD patients has been reported to be between 1.5% to 4.5% [23] that is lower than that of our study. This difference is presumably due to the low number of patients studied in our study and hence we recommend further investigations with more patients in region. In addition, in this study, over 6.7% of patients had relative shortage of AAT. In healthy controls, no definite and relative shortage of this enzyme has been found. There is no report from Iranian patients to be inconsistent or consistent with our study. In a study done by Dahl and his colleagues in 2002 on 9170 matures, performed randomly on normal population, it was shown that the prevalence of this deficiency in the Northern Europe was at the level of definite shortage of AAT, and the researchers proposed that with the presence of other risk factors of COPD, these people will suffer from severe COPD in their life [31]. In another study conducted by Rodriguez et al. (2002), it was identified that people with AAT lower than normal, even in the absence of the risk factors of COPD, suffered from this disease more than normal people [32]. None of these two studies examined the amount of ATT in COPD patients.

Our results showed that there are significant differences between frequency of AAT deficiency and severe COPD compared to the healthy group without explanation of

demographic and environmental agents associated with the disease especially smoking exposure that can exerts a significant modifier effect on disease intensity ( $P=0.001$ ). There is also no comparison of ATT deficiency available in severe COPD patients, so more studies are needs to be designed to better identify the phenomena of ATT deficiency in severe COPD patients. On the other hand it can be said that although our study did not concern itself with genetic associations leading to the deficiency of ATT in COPD patients, as Stockley has suggested, we can provide a strategy for early detection of this protein in the serum and changing of patients' lifestyle [21].

## Conclusion

This study showed that the prevalence of definite shortage of ATT in severe COPD patients in Zahedan is 10% thus being higher than the anticipated amount in European countries and the amount of relative shortage

as 6.7% that is almost similar to that of European countries. In these patients, several risk factors have been effective like smoking and shisha, occupational pollution like agriculture and baking bread. Also according to the results of this study, no cases of definite and shortage of ATT deficiency were found in healthy control group. A significant difference was found between the patients and the healthy group in terms of frequency of this enzyme that is one of the involved factors in severe COPD patients and likely a reason for high prevalence of severe COPD and its severity in Zahedan.

## Conflict of Interest

There is no conflict of interest to declare

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