Journal of Kerman University of Medical Sciences

JKMU

Journal of Kerman University of Medical Sciences, 2017; 24(2): 103-110

Prevalence of Alpha-1 Antitrypsin (A1AT) Deficiency among Patients with COPD in Kerman, Iran

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Received: 19 May, 2016 Accepted: 27 February, 2017

ARTICLE INFO

Article type:
Original article

Keywords:
Alpha-1 Antitrypsin (A1AT)
Chronic Obstructive Pulmonary Disease (COPD)
Prevalence

Abstract

Background: One of the genetic risk factors for chronic obstructive pulmonary disease (COPD) is deficiency of Alpha-1 Antitrypsin (A1AT). There is no exact statistics about the prevalence of this disease in different regions of Iran. The present study aimed to determine the prevalence of alpha-1 antitrypsin (A1AT) deficiency in COPD patients in Kerman, Iran.

Methods: In the present study, the serum level of AAT in 294 COPD patients visited in the pulmonary clinic center in Kerman, Iran was measured. The diagnosis of COPD was confirmed through history taking and Spirometry before and after using Bronchodilator. Data analysis was done by using t-test and Chi-square test.

Results: Among294 studied patients, 223 individuals (75.9%) were male. None of the patients had absolute deficiency of A1AT, and only13 patients (4.4%) had a relative deficiency of A1AT. There was a statistically significant relationship between relative deficiency of A1AT and the severity of COPD in a way that in most of the cases, relative deficiency of this enzyme was associated with severe and very severe airway obstruction.

Conclusion: Based on the results of the present study and other similar studies in Iran, absolute and relative deficiency of A1AT in Iran has less frequency compared to other regions of the world and other factors such as cigarette smoking, opium addiction and consumption of fossil fuels have a more significant role in the prevalence of COPD in Iran.

Citation: Samareh Fekri M, Hashemi Bajgani S.M, Jamshidi Gohari N, Bazargan Harandi N, Rahmatian M. Prevalence of Alpha-1 Antitrypsin (A1AT) Deficiency among Patients with COPD in Kerman, Iran. Journal of Kerman University of Medical Sciences, 2017; 24(2): 103-110.

Introduction

Chronic obstructive pulmonary disease (COPD) is one of the most important causes of mortality in the world and the main cause of one third of deaths in the European Union. It should be noted that an increase in death is predicted for future decades (1-3). Chronic obstructive pulmonary disease (COPD) as presumed in global initiative for chronic obstructive lung disease (GOLD) is defined as a preventable and treatable disease with extra pulmonary effects which might lead to severe disease condition in certain patients. This disease originates in lungs generating irreversible and progressive obstruction of airways and abnormal inflammatory response of lungs to gas and suspended particles and consequently leading to diseases such as chronic bronchitis and emphysema (4-5). The main cause of this disease is the combination of genetic and environmental factors. More than 90% of COPD patients are current smokers. Therefore, it is apparent that the living environment plays a significant role in the disease progress. Although the effect of cigarette on lung function varies, the role of genetics in the incidence of this disease along with cigarette smoking has been considered (6-7). Frequent change of the expression of Alpha-1 Antitrypsin (A1AT) gene, as a preventive protease in the circulatory system, is the only known factor which causes chronic obstructive pulmonary disease (8-9). Alpha-1 Antitrypsin (A1AT) is an acute-phase protein, which is mainly produced in the liver. This protein protects lung from destruction by Neutrophil elastase (10-11). The deficiency or dysfunction of Alpha-1 Antitrypsin (A1AT) leads to protease – anti-protease imbalance in the lung and enhances emphysema (12). The deficiency of this enzyme in cirrhosis of the liver, primary hepatic carcinoma and some other types of vasculitis show the significance of this enzyme and its protective role (13-14).

The most common form of AATdeficiency is the ZZ hemozygote type in whichthe serum level of this glycoprotein decreases to 10-15% of the normal level. Its protective level is about 11 mMOL/lit and emphysema occurs in the levels less than 9 Mmol/lit. The screening of AAT is recommended in patients with COPD, emphysema, asthma with irriversible obstruction of airways, necrotizing paniculitis and inneonates and children with liver disease and also in the family of the patients with hemozygote AAT (15).

It is estimated that just 5% of the patients with AATD are diagnosed in the united states, so most of patients are unaware of their disease and it takes about 8.3±6.9 years between appearing the symptoms of pulmonary disease and the diagnosis and 30.8% of the patients are diagnosed in the ages more than 50 years(16) on the other hand, since pulmonary symptoms of AAT deficiency are similar to asthma or COPD and the first symptoms are shortness of breath, cough, increase of sputum, decrease of ability in activities and wheezing, the correct diagnosis is delayed. These patients need A1AT therapy in addition to bronchodilator inhalers, corticosteroids and smoking quit is also recommended (16).

In a study on a large number of severe asthma patients assumed to have irreversible airway remodeling, measuring the A1AT level in serum revealed that these patients had A1AT deficiency, while they had been misdiagnosed as asthma patients (17).

Because of the ambiguity in the diagnosis of A1AT deficiency, increase in the frequency of chronic obstructive pulmonary disease (COPD), its significance, treatment costs and mortality in the public health system, and role of deficiency of Alpha-1 Antitrypsin (A1AT) in the incidence of COPD, as well as lack of comprehensive information about this disease in Iran, the present study aimed to determine the

prevalence of Alpha-1 Antitrypsin (AAT) deficiency in COPD patients in Kerman, Iran.

Methods

In the present descriptive and cross-sectional study, 294 COPD patients visited in the pulmonary center of Besat clinic affiliated to Kerman University of Medical Sciences were studied. After describing the objective of the present study and taking informed consent from patients, based on the ethical code of 141/92KA as received from the ethical committee of Kerman University of Medical Sciences, patients with clinical symptoms of COPD underwent spirometry test. Those with pulmonary functionofFEV1/FVC≤0/7 and those who had less than 12 percent and 200cc increase of FEV1 after applying 2 puffs of salbutamol for the next 15 minutes were diagnosed as COPD cases and were categorized into four groups of mild (FEV1 ≥ 80%), moderate (80%> FEV1≥50%), severe (50%>FEV1≥30%) and very severe (FEV1<30%)(18). Exclusion criteria were acute and chronic inflammatory diseases, infections, cancer, taking OCP, pregnancy, severe stress and diseases with decreased serum proteins such as malnutrition and nephrotic syndrome. Then, 5 ml of venous blood was obtained from each patient. After centrifuging and freezing of the serum, it was kept in -20 ° C. When the sampling was completed, all obtained samples were analyzed by Nephelometry method and MININEPH kit made in Germany for measuring A1AT level. The kit was put in 400 micro liters of diluted buffer (1:11 dilution) and 40µlA1ATantibody. The level of turbidity generated by antigen-antibody complexes were read by MININEPH Nephelometry system. The values were obtained in g/l scale and measured with an accuracy of 0.16 g/l. Due to application of Nephelometry method in this laboratory kit, values which were less than 1 g/l were termed as relative deficiency while those which were less than 0.5 g/l were termed as absolute deficiency.

The data derived from forms filled in by patients, such as age, sex, history of cigarette smoking and using opium, history of baking, occupation, using drugs, co-morbidities and severity of disease were analyzed through SPSS software (version18).

Results

In the present study, 294 individuals had COPD in Spirometry analysis, of whom 223 individuals were male (75.9 %). Mean age of these patients was 62.02±11 years with an age range of 26 to 90 years old. Among the diagnosed patients, 128 patients (43.5 %) were current smokers, 82 were ex-smokers and 84 participants (28.6%) had never smoked before. In smokers, mean number of smokedcigaretteswas25.19±16pack /year. From all, 203 patients (69%) had history of opium addiction and 62 patients (21.1%) had history of baking. Among the studied COPD patients, based on the classification of GOLD criteria, 19 individuals (6.5%) had mild disease, 125 individuals (42.5%) had moderate disease, 100 individuals (34%) had severe disease and 50 individuals (17%) had very severe disease. Mean serum level of A1AT was 0.37±1.57g/l ranging from the minimum of 0.54g/l to maximum of2.90 g/l. None of the analyzed A1ATdeficient patients had an absolute deficiency, but 13 patients (4.4%) had a relative A1AT deficiency. There was a statistically significant association (P=0.0070) between relative deficiency of A1AT and severity of COPD disease (table 1 and fig. 1). Mean percentage of FEV1 in Spirometry of patients with relative A1AT deficiency was 37.92±23.90 l/s. However, it was 50.17±19.95 l/s in patients without deficiency so that the difference in these two groups was statistically significant (P=0. 033).

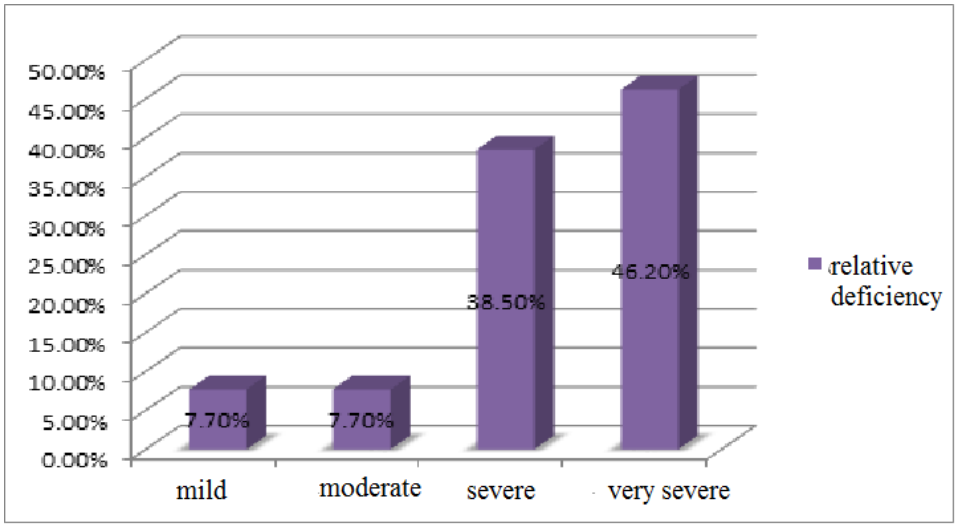

Figure 1. Frequency of Alpha-1 Antitrypsin (AAT) Deficiency Based on the Severity of COPD

There was no statistically significant association between the frequency of relative A1AT deficiency and the consumption of Theophylline (P=0. 331), short-acting beta-agonists (P=0. 085), long-acting beta-agonists (P=0. 265), inhaled corticosteroids (P=0. 313), systemic corticosteroids (P=0. 452), proton pump inhibitors (P=0. 834), ipratropium bromide (P=0. 850) and H2 blockers (P=0. 850). There was no statistically significant relationship between the relative deficiency and diseases such as diabetes mellitus (P=0. 210), hypertension (P=0. 111), coronary artery disease (P=0. 509), renal failure (P=0. 722), Hypercholesterolemia (P=0. 210) and gastro-esophageal reflux (P=0. 976). The results have been shown in table2. As it is seen in table 3, there was no significant association between occupation and A1AT deficiency (P=0. 959) and as table 4 shows, A1AT deficiency had no significant association with body mass index (P=0. 075), age (P=0. 627), sex (P=0. 975), cigarette smoking (P=0. 902), opium addiction (P=0. 181) and baking (P=0. 541).

Table 1. The relationship between the frequency of Alpha-1 Antitrypsin (A1AT) deficiency and the severity of COPD.

Severity of COPD	AAT≤1g/l	AAT>1g/l	P Value
Mild	1 (7.7)	18(6.4)	
Moderate	1(7.7)	124(44.1)	0.007
Severe	5(38.5)	95(33.8)	
Very Severe	6 (46.2)	44 (15.7)	

P-value were estimated in a logistic regression, AAT= Alpha-1 Antitrypsin, COPD=chronic obstructive pulmonary disease

Table 2. The relationship between the frequency of Alpha-1 Antitrypsin (AAT) deficiency and history of previous diseases

Disease	AAT≤1g/l	AAT >1g/l	Total	P-Value
Diabetes Mellitus	0 (0)	32 (11.6)	32 (11.6)	0.210
Blood Pressure	1 (7.7)	73(26.4)	74(25.6)	0.111
CAD	2(15.4)	55(19.9)	57(19.7)	0.509
Renal Failure	0(0)	7(2.5)	7(2.5)	0.722
Hypercholesterolemia	7(2.5)	7(2.5)	7(2.5)	0.210
GERD	5(38.8)	107(38.8)	112(38.8)	0.976

P-value were estimated in a logistic regression, AAT= Alpha-1 Antitrypsin

Table 3. The relationship between the-frequency of Alpha-1 Antitrypsin (AAT) deficiency and occupation

Occupation	AAT≤1g/l (Per) No	AAT >1g/l (Per) No	Total	P Value
Baker	0(0)	1(0.4)	1(0.3)	0.959
Farmer	2(15.4)	50(17.9)	52(17.8)	
Pitman	0(0)	2(0.7)	2(0.7)	
Steel Factory Worker	0(0)	16(5.7)	16(5.5)	
Miner	0(0)	16(5.7)	16(5.5)	
Tire Industry worker	0(0)	1(0.4)	1(0.3)	
Employee	2(15.4)	38(13.6)	40(13.7)	
Cement Factory Stuff	0(0)	4(1.4)	4(1.4)	
Carpet Maker	0(0)	14(5)	14(4.8)	
Welder	0(0)	7(2.5)	7(2.4)	
Rancher	0(0)	5(1.8)	5(1.4)	
Painter	0(0)	2(0.7)	2(0.7)	
Other	9(69.2)	123(44.1)	132(45.2)	

P-value were estimated in a logistic regression, AAT= Alpha-1 Antitrypsin

Table 4. The relationship between the frequency of Alpha-1 Antitrypsin (AAT) and selected baseline characteristics

Characteristics		AAT≤1g/l	AAT >1g/l	Total	P- Value
Sex	Male	10(76.9)	213(75.8)	223(75.9)	0.975
	Female	3(23.1)	67(23.8)	70(23.8)	0.975
	Negative	3(23.1)	81(28.8)	84(28.6)	0.902
Cigarette Smoking	Ex-smoker	4(30.8)	78(27.8)	82(27.9)	0.902
	Currently	6(46.2)	122(43.4)	128(43.5)	0.902
Bakery	Negative	10(76.9)	222(79)	232(78.9)	0.541
	Positive	3(23.1)	59(21)	62(21.1)	0.541
Opium	Negative	6(46.2)	85(30.2)	91(31)	0.181
Addiction	Positive	7(53.8)	196(69.8)	203(69)	0.181
Age		62.84±9.8	61.20±11.9		0.627
BMI		21±2.8	22±5.8		0.975

P-value were estimated in a logistic regression, AAT= Alpha-1 Antitrypsin

Discussion

In the present study, there was no case of absolute A1AT deficiency, but 4.4 % of the studied patients had relative A1AT deficiency. There was a statistically significant association between the relative deficiency of A1AT and the severity of COPD and FEV1, so that most of the cases of relative A1ATdeficiency were observed in the patients with severe and very severe airway obstruction.

According to a study in Denmark done randomly on adult individuals, the prevalence of different alleles deficiency of A1AT gene was less than 1% andA1ATdeficient individuals were the COPD patients with lower ages; however, there was no significant association between the age and deficiency of this enzyme (19). Similar to our study, the mentioned study showed a significant association between the severity of disease and deficiency of A1AT.

Another study on COPD patients in USA showed that the prevalence rate of0.63 % for homozygote type of A1AT deficiency and 10.88% for heterozygote type. It showed a significant association between cigarette smoking and AAT deficiency, but there was not a significant association between severity of disease and deficiency of A1AT (20). However, our study revealed that there is no significant relationship between cigarette smoking and A1AT deficiency while there is a significant relationship between severity of disease and A1AT deficiency. These discrepancies can be attributed to race, geographical and the sample size differences in these two studies.

Another study in Ireland showed that the prevalence of Alpha-1 Antitrypsin (A1AT) deficiency is 4%forheterogeneous type and 10%for homogeneous type which is higher than other countries (21). A study in the United States estimated the deficiency of this enzyme to be 1:3000 at national scale (22). A study in Poland showed that the level of absolute Alpha-1 Antitrypsin (A1AT) deficiency in COPD patients is similar to that of the European Union (i.e. 1.45%). That study showed that the prevalence of A1AT deficiency is higher in older individuals (23). According to a study in Argentina on COPD patients, the prevalence of AAT deficiency was 2.1 %, which is similar to the Southern European countries due to Italian-Spanish background of Argentina population (24). In a study in Portugal on COPD patients, the frequency of A1AT deficiency was 4.1%, while this value was 1.87% for COPD patients living in Lithuania (25, 26).

The prevalence of COPD in Spain is reported to be 10.2%. If cigarret smoking wasthe main ethiologic risk factor of COPD, use of biomass and AATD would berelated to this disease too. AATD is a genetic disorder which can cause 2-3% of the COPD cases. In a study on 80 COPD patients in Spain in 2016, 30% of 40-80 years old patients were carriers of heterozygote AATD and there was no case of severe deficiency of hemozygute PIZZ, and the allelic repeat of antithrypsin gene was reported to be 3.1% for PIZ using PCR, although the population has been Galician population (27). Similarto our study, there were no hemozygote case in the mentioned study. The main reason for more heterozygote cases could be attributed to the smallsample size and race.

In a study in Saudi Arabia on 155 COPD patients, the frequency of absolute A1AT deficiency was 11.1% which was much higher than that in Iran (i.e. 4.4). This difference can be due to race, genetic or sample size differences (28).

Another study was found that the frequency of deficiency of A1AT enzymes in Iran, Pakistan, Afghanistan and Tajikistan is higher than in other Asian countries (29). However, by considering the sample size of our study, it seems that the prevalence of absolute A1ATD cases in Iranian society, with a combination of Arab and Asian races, is lower than the European population (i.e. 1.5-4.5%)(30).

With respect to this issue, there are limited studies. In a study in Hamedan, Iran in 2007 on 125 COPD patients, there was no case of absolute Alpha-1 Antitrypsin (A1AT) deficiency while the frequency of relative deficiency was reported to be 20% (13). In the mentioned study, no significant association between the severity of disease and

relative deficiency of A1AT enzyme has been reported, while in our study a significant association between these two factors was observed. These different sets of findings might be due to the difference in sample size. Similar to the present study, there was no significant association between deficiency of A1AT enzyme and sex, occupation and cigarette smoking. In another study in 2010 on130 COPD patients and 50 normal individuals in Shiraz, there was no case of absolute deficiency of A1AT among normal individuals and the percentage of relative A1AT enzyme deficiency was not reported (31).

Conclusion

In the present study, there was no case of absolute A1AT deficiency, but the prevalence of relative A1AT deficiency was 4.4 %in the studied patients. There was a statistically significant association between the relative deficiency of A1AT and severity of COPD and FEV1, so that most of the cases of relative A1AT deficiency were observed in the patients with severe and very severe airway obstruction.

Based on the results of this study, the main reason of high prevalence of COPD in Iran was not the deficiency of Alpha-1 Antitrypsin (A1AT), but is cigarette smoking, addiction to opium, occupational pollutions and history of baking. Therefore, national health system should provide more guidelines and educational courses for the abandonment of cigarette smoking and opium consumption as well as arrangements for correct baking and improvement of health conditions in work environments and industrial factories by sufficient investment.

Limitations

If we had a larger sample size, cases of absolute deficiency of Alpha-1 Antitrypsin might have been found and a significant association between cigarette smoking, opium use, age and deficiency of A1AT could be statistically supported.

Acknowledgement

The authors appreciate the financial support of Kerman University of Medical Sciences and contributions of staff of Besat clinic.

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