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Original article

Evaluation of alpha-1-antitrypsin levels in blood serum of patients with chronic obstructive pulmonary disease

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Summary. *Introduction:* Chronic obstructive pulmonary disease (COPD) is a disease that causes obstructed air flow from the lungs. The disease also has a dramatic role in increasing rate of mortality and morbidity in recent years. Air pollution, long-term exposure to particulate matter and irritating gases, especially cigarette smoke, genetic inheritance which has an impact on the initial forced expiratory volume one in second (FEV_1), and alpha-1-antitrypsin (AAT) deficiency are among common COPD risk factors. The objective of this study is to evaluate parameters and serum AAT levels in COPD patients. *Materials and Methods:* Having taken the approval of local ethical committee, this cross-sectional study was performed with adult patients diagnosed with COPD, whose serum AAT levels were measured through nephelometric analysis in Kars Harakani State Hospital where secondary health care is served. The study evaluated ATT levels in patients' serum in relation to their age, gender, body mass (BMI), exposure to cigarette smoke, FEV_1 percentage, hospitalization in pulmonology or intensive care unit through a year, mortality status, white blood cell (WBC), c-reactive protein (CRP) and blood gases. *Results:* The average age of the 243 patients included in the study was 68.41±11.52 and 160 (65.8%) of them were male. The age and BMI of the female patients were higher. Of the all patients only a single patient's serum AAT level was below the reference value. AAT levels were similar in both genders irrespective of their being exposed to cigarette smoke or being discharged or being exitus at their first admission to hospital, being exitus in the first year of disease diagnose, and being hospitalized in intensive care unit. AAT levels were reasonably correlated with WBC and CRP in a positive way ($p<0.001$ $r=0.289$ for WBC; $p<0.001$, $r=0.295$ for CRP). AAT levels were seen to significantly increase along with COPD stages which go up with FEV_1 percentages ($p<0.001$). CRP was watched to have increased to Stage III COPD (severe COPD). However, it was watched to have decreased in Stage IV (very severe COPD) ($p=0.179$). *Conclusion:* In the study, AAT serum levels of COPD patients were examined. The levels and their relations in various parameters of the patients were evaluated. (www.actabiomedica.it)

Key words: serum alpha-1 antityrpsin level, chronic obstructive pulmonary disease

Introduction

COPD is a substantial reason of morbidity and mortality world-wide (1). COPD is the 5th most common cause of death and also 10th heaviest burden disease according to the World Health Organization (2). COPD is one of the chronic diseases which have critically increased mortality in recent years. Global Initiative for Chronic Obstructive Lung Disease (GOLD) describes the disease as: it is a disease which increases patients' clinic severity, which bears significant extrapulmonary effects, and which can be prevented and cured. It causes not fully reversible airflow limitation in lungs (1). The airflow limitation caused by COPD

is usually progressive and the limitation is related to the abnormal inflammatory response in lungs, which is caused by noxious particles or gases (1). Even if it may differ in general population, the risk factors are divided into two as endogenous or host and exogenous or environmental. (1). Air pollution, long-term exposure to particulate matter and irritating gases especially cigarette smoke, genetical inheritance, having an impact on FEV1, and AAT, the major serum serine protease inhibitor are known risk factors for COPD (1).

AAT is a well-known glycoprotein which weighs 52-kDa and includes three N-glycosidically linked complex oligosaccharides and belongs to the serpin family (3). AAT automosol dominant is inherited and, coded by SERPINA1 gene placed in the long arm of 14th (14q31–32.3) chromosome (4). AAT variations are divided into four groups: a) Levels of common alleles, normal variants and nephelometric serum are between 80-220 mg/dL; b) in deficient variations, nephelometric serum levels are below and in some alleles functional activity of AAT molecule are reduced; c) rare alleles are null variations characterized by AAT, which exists in serum as a result of transcriptional or translational errors in protein synthesis; d) dysfunctional variants are characterized with abnormal function of AAT (4). AAT deficiency is most frequently identified with nephelometric measurement especially serum levels are below 100 mg/dL (4).

The role of AAT is to neutralize neutrophil elastase enzyme and to preserve connective tissue of the lung from degeneration by elastase released from neutrophils (3). AAT is an acute phase reactant produced by mainly liver, macrophages and monocytes whose concentration amount increases also in plasma during malignancy, inflamination and infection (3). Plasma level of AAT in a healthy individual is between 0,9-2 g/L (5). However, it may multiply 4 to 5 times in some cases such as acute inflammation and infection (5). Apart from its acute phase response, it increases in the third trimester of pregnancy and in a parallel way to ageing (5). Deficiency of functional AAT may cause early beginning emphysema, bronchial asthma, bronchiectasis, panniculitis, systemic vasculitis, spontaneous cervical artery dissections, type 2 diabetes mellitus, spontaneous abortions and human immunodeficiency virus (HIV) type 1 (5).

The objectives of this study are to evaluate age, gender, BMI, exposition to tobacco, smoke, percentage of FEV_1 and degree of airflow obstruction in relation to those percentages, hospitalization in pulmonology and intensive care unit in a year, mortality status, WBC, CRP and blood gases of COPD patients in a secondary service hospital; and to evaluate serum AAT levels measured through nephelometric analysis in relation to patients' parameters.

Materials and methods

This cross-sectional study was conducted with the approval of the local ethical committee, between the dates 01.01.2013-31.12.2015, in adult patients diagnosed with COPD in Kars Harakani State Hospital, Kars, Turkey.

People who were included in the study are; adult patients formerly diagnosed with COPD and present to the emergency room or pulmonology with any reason as ambulant between the specified dates or hospitalized to intensive care unit, and adults whose serum AAT was examined as external examination. Patients who hadn't been diagnosed with COPD before admission to hospital and ones whose serum AAT levels weren't examined were not included in the study. Data belonging to patients; a) age, gender, length (metre), kilo (kilogram), BMI (kilo/length), exposition to tobacco smoke ; b) percentage of FEV_1, measured with spinometer after bronchodilator and degree of airflow limitation in relation to those percentages (Stage I: Mild- FEV_1 percentage ≥80%, Stage II: Moderate-50% ≤FEV_1 <80%, Stage III: Severe-30% ≤ FEV_1 <50%, Stage IV: Very Severe-FEV_1 <30% (6)); c) number of hospitalization to pulmonology and history of hospitalization to intensive care unit, result of existing admissions (discharge or exitus) and to be mortality in a year; d) reference WBC (10^3/ul), reference CRP (mg/L), reference pH obtained from blood gas, partial pressure of oxygen (PaO_2-mmHg), partial pressure of carbon dioxide ($PaCO_2$-mmHg); e) serum AAT level measured with nephelometric analysis (mg/dl) (reference levels 88-174 mg/dL(Ankalab Laboratories, Ankara, Turkey) were evaluated. Data obtained from the study were analyzed through "SPSS for Windows ver.

22.0" packet program. Continuous variables average ± with standard deviation and frequency data (%) were described with numbers. In statistical analysis, the convenience of all measurable variables to normal distribution was evaluated with histogram and Kolmogorov-Smirnov test. Since data didn't adjust to normal distribution, "Kruskal Wallis Test" was used in comparison of more than two groups. In doublet comparison of groups, "Bonferroni correction Mann-Whitney U Test" was used. Relations between variables were evaluated with analysis of Sperman Correlation. In comparison of data gained through enumeration, "Chi square test" , "Fisher's exact test" and "Linear- by-linear association" were used. Existence of dependent effects of hospitalization to intensive unit care and mortality variables was evaluated with "Binary Logistic Regression" analysis. Value of foreseeing AAT levels in hospitalization to pulmonology and intensive unit care and one-year-mortality was done with ROC analysis. In all tests, level of statistical relevance was accepted as $p<0.05$.

Results

Average age of the patients was 68.41±11.52 and distribution of ages was seen to be normal. 160 (65.8%) of the patients were male. Weight values of the both male and female patients were similar but levels of age and BMI were higher in females whereas length level was shorter (Table 1).

Only one of the 243 patients included in the study had 25,5 mg/dL level of serum nephelometric AAT and it was below the value of reference. AAT levels were 205.08±58.09 in females, and 205.08±58.09 in males. The levels were similar in both genders (p=0.426). AAT levels of patients exposed to tobacco smoke were 210.61±59.18, whereas AAT levels of patients not exposed to tobacco smoke were 205.15±58.73. The levels were similar in both groups (p=0.214). AAT levels of patients who were discharged from hospital at their existing admission were 208.75±59.15, whereas they were 198.33±53.96 in exitus patients. The levels were similar in both groups (p=0.709). AAT levels in patients who became exitus in the first year were 209.04±58.89 while they were 208.14±59.06 in non-exitus patients. The both groups were similar (p=0.387). AAT levels of patients who were hospitalized to intensive unit care in a year were 207.44±52.32 whereas levels of patients not have been hospitalized to intensive unit care were 208.50±60.24. They were similar in both groups (p=0.491).

Table 1. Distribution of age, length, weight and BMI as to gender

	Kadın	Erkek	p
Age, (year)	71.53±10.84	66.79±11.57	**0.003**
Length , (m)	1.54±0.12	1.64±0.15	**<0.001**
Kilo, (kg)	71.60±19.09	71.43±18.04	0.974
BMI, (kg/cm^2)	30.35±8.44	27.37±11.06	**<0.001**

AAT level was correlated with WBC and CRP in a positive way ($p<0.001$, $r=0.289$ for WBC; $p<0.001$, $r=0.295$ for CRP). It was seen that AAT levels significantly increased along with COPD stages which rise in regard to percentages of FEV1($p<0.001$). However, it was seen that CRP increased till Stage III and decreased at Stage IV ($p=0.179$).

Discussion

AAT is a protein which inhibits neutrophil elastase, whose deficiency is hardly seen and prevalence of its deficiency shows variety as regard to population study (7). Patients usually get the diagnosis of AAT deficiency at approximately 45 and unfortunately almost 85% of patients don't have a diagnosis along all their lives (7). One of the patients included in the study had serum nephelometric level which was below the range of reference value. The patient was a 53-year-old tobacco user. Considering the low serum level of the patient, the patient was thought to have AAT deficiency and genotypic analysis was done. Homozygous allel PI*ZZ was identified in the patient. Similar to the age and gender of this patient, in the study of Piras et al, AAT deficiency in males was higher in smokers and the age of diagnosis of this deficiency was approximately 47±14.7 (8). In the study age average of COPD patients was 68.41±11.5 and distribution of ages was normal. Most of the patients were male (65.8%). In the studies of Zillmer et al and Barrecheguren et al, the

Table 2. FEV_1 percentages and demographic features of the patients as to COPD stages and laboratory data

	Stage I (n=9, 3.7%)	Stage II (n=57, 23.5%)	Stage III (n=117, 48.1%)	Stage IV (n=60, 24.7%)	P
Age (Year)	64.78±12.48	65.32±13.89	70.93±10.35	66.97±10.17	0.007*
Gender					0.241**
Female	3 (33.3%)	22 (38.6%)	42 (35.9%)	16 (26.7%)	
Male	6 (66.7%)	35 (61.4%)	75 (64.1%)	44 (73.3%)	
Length (m)	1.61±0.13	1.58±0.15	1.60±0.16	1.65±0.12	0.062*
Kilo(kg)	73.78±13.25	73.40±19.73	72.12±18.68	68.08±16.95	0.371*
BMI(kg/m^2)	29.11±7.62	29.85±9.65	29.13±11.92	25.43±6.99	**0.048***
Levels of AAT	193.89±45.95	199.44±59.29	211.01±63.16	212.22±51.26	**<0.001***
Negatif	-	-	1 (0.9%)	-	0.097**
Reference range	3 (33.3%)	22 (38.6%)	31 (26.5%)	14 (23.3%)	
Positive	6 (66.7%)	35 (61.4%)	85 (72.6%)	46 (76.7%)	
Exposure to tobacco					0.660**
Yes	6 (66.7%)	27 (47.4%)	58 (49.6%)	34 (56.7%)	
No	3 (33.3%)	30 (52.6%)	59 (50.4%)	26 (43.3%)	
WBC	7.89±1.42	10.47±4.88	10.38±5.15	10.69±4.11	0.245*
CRP	1.61±1.17	3.70±4.13	5.49±7.12	5.22±6.61	0.179*
pH	7.43±0.08	7.42±0.06	7.42±0.08	7.40±0.11	0.419*
pCO2	41.22±8.39	41.06±9.47	43.25±11.48	47.54±12.29	0.056*
pO2	46.67±12.12	49.56±14.77	46.09±11.39	43.10±13.44	0.194*
Number of hospitalization in a year, median	1±0.87	1.84±1.66	2.52±2.42	2 (0-15%)	**0.024***
Exitus in hospital					**0.049****
Yes	-	1 (1.8%)	3 (2.6%)	5 (%)	
No	9 (100%)	56 (98.2%)	113 (97.4%)	54 (91.5%)	
Exitus in the first one year					0.193**
Yes	2 (22.2%)	7 (12.3%)	35 (23.7%)	14 (23.7%)	
No	7 (77.8%)	50 (87.7%)	81 (69.8%)	45 (76.3%)	
Hospitalization to intensive unit care in the first one year					**0.008****
Yes	-	5 (8.8%)	24 (20.7%)	15 (25.0%)	
No	9 (100%)	52 (91.2%)	92 (79.3%)	45 (75.0%)	

* Kruskall-Wallis test; **Ki-kare test

age average of the patients was 68.5±9.6, 52.6±16.3. 45.3% and 55.5% of the patients were male (9, 10).

Even though in the study the number of the male patients was more than the females, the age average and BMI of the females were higher. Similarly, in the study of Harik-Khan et al done with COPD patients, at first admission of the females to the hospital, age and BMI respectively was 55.4±4.2 and 25.4±3.5, which was higher when compared to the males (11). Different from all those studies, Senn et al, Ferrarotti et al studied the relation of serum AAT with BMI; and Senn et al showed that there occurred a negative relation between those two parameters whereas Ferrarotti et al displayed that AAT didn't get affected from age, gender and BMI (12,13). In this study, it was released that AAT levels in both genders were seen to be similar in patients no matter they were exposed to tobacco or not. It was seen that serum level which belongs to this protein wasn't affected from gender and exposure or non-exposure to tobacco. In the study of Barrecheguren et al, approximate serum AAT level was 150.9±34.2 and that was higher in smokers compared to former smokers, which

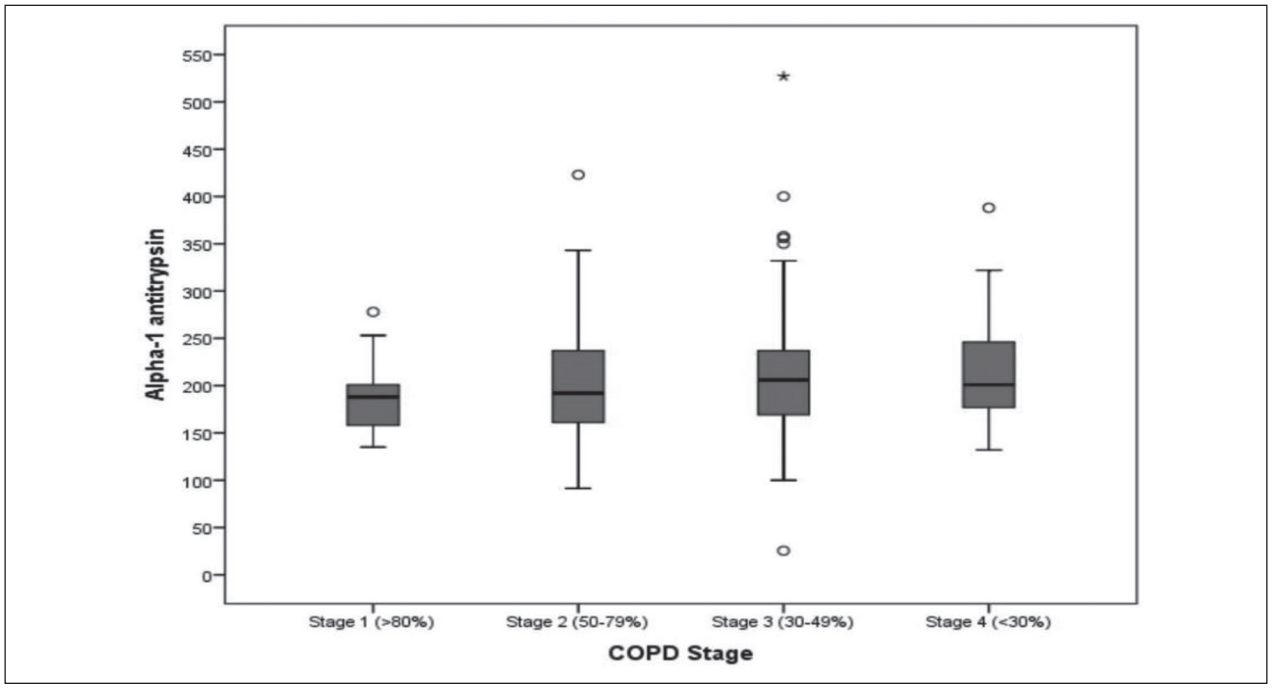

Figure 1. Box plot graphs AAT and COPD Stage

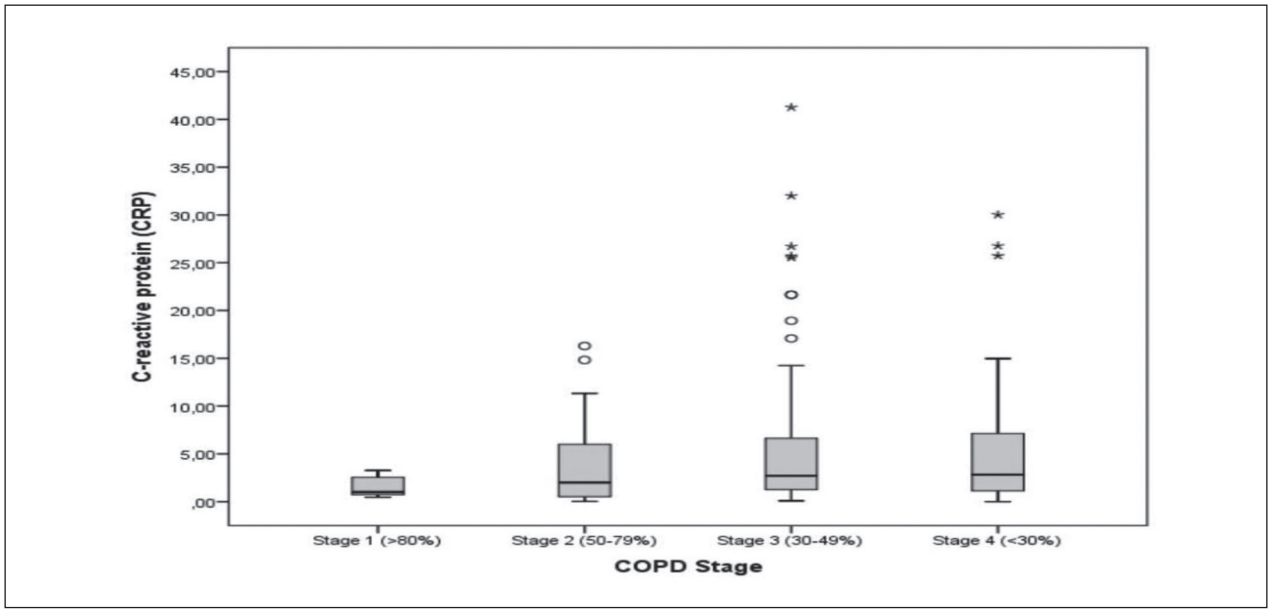

Figure 2. Box plot graphs of CRP and COPD Stage

showed that exposure to tobacco smoke affected level of AAT (10). In the studies of Senn et al, AAT levels were positively related to males and postmenopausal female age group; it depended on rising dose in patients with exposure to tobacco (12). Serum AAT level in the study of Ferrarotti et al didn't get affected from smoking status (13). In the study, AAT levels were similar between groups of patients who were discharged from

hospital or became exitus at their existing admission to hospital, who became exitus or not in the first year, who were hospitalized to intensive care unit or not in a year. Those results showed that AAT serum levels didn't affect to be discharged from hospital, mortality in a year and hospitalization to intensive unit care. In the studies of Nuijens et al, serum elastase-AAT complex was an significant prognostic factor and was higher in groups of patients who became exitus than patients who survived (14). The AAT Deficiency Registry Study Group informed that serum levels which increased with intravenous AAT augmentation therapy increased survivability (15). In the studies of Li et al, concentration of serum AAT significantly affected (16). In the studies of Sclar et al, serum AAT levels which increased with augmentation therapy provided longer life span (17). Carey et al showed that after liver transplantation, patients with AAT deficiency displayed normalizing AAT serum levels and together with that the rate of survivability for a year increased to 90 % (18). On contrary to those studies, in the studies of Simpsons et al, survivability day was 300 days more in the group with low level of AAT. (19).

In this study, serum AAT level was reasonably correlated in a positive way with WBC and CRP, which is a marker of inflammation. In studies of Nuijens et al, serum elastase-AAT complexes levels weren't correlated with number of WBC and on the contrary, AAT level was significantly high in patients whose WBC number was low (14). According to Senn et al, factors which are related to serum AAT levels didn't get affected from CRP and CRP itself was positively related to Serum AAT level (12). According to Ferrarotti et al, serum AAT levels didn't get affected from CRP levels (13).

Since the beginnings of 1980, patients with AAT deficiency have been given purified human AAT concentration and their serum levels have been increased. Additionally, in those patients whose serum levels were increased and cured, it was seen that there was a less decrease in their FEV_1percentages (20). In the study, it was seen that approximate AAT levels significantly increased along with seriousness of COPD stages which raised percentages of FEV_1. CRP increased till Stage III and it decreased at Stage IV. According to Senn et al, since there was an absence of adjustment, serum AAT level was inversely associated with FEV_1 (12). Serum levels which increase with The AAT Deficiency Registry Study Group intravenous AAT augmentation therapy didn't have a general affect on FEV_1 decrease ratio. However, patients predicted with FEV_1values of 35 to 49% were stated to have a slower decrease in values (15). According to Sclar et al, predicted FEV_1 which increased with AAT augmentation therapy was decreasing (17).

Conclusion

In the study, in a secondary care serving hospital where limited examinations can be done, AAT serum levels as external examination of patients diagnosed with COPD were studied. Only one of the patients included in the study was seen to have AAT deficiency and genotypic analysis was done. Relations of AAT serum levels and relations of parameters belonging to patients were evaluated. The limitations of the study are not to be able to reach laboratory results of some patients, and to be able to measure only serum AAT levels. Studies in which especially AAT levels are examined and advanced research to be done are significantly needed.

Conflict of interest: None to declare

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