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Relation of functional characteristics and serum alpha-1-antitrypsin (AAT) concentration in patients with PiMM phenotype and chronic obstructive pulmonary disease (COPD)

Bojan Zarić [a,*], Jelena Stojčević [b], Ljiljana Andrijević [c], Ilija Andrijević [a], Vesna Kuruc [a], Igor Veselinović [d], Iva Salatić [d]

[a] Institute for Pulmonary Diseases of Vojvodina, Faculty of Medicine, University of Novi Sad, Serbia
[b] Center for Laboratory Medicine, Clinical Centre of Vojvodina, Faculty of Medicine, University of Novi Sad, Serbia
[c] Oncology Institute of Vojvodina, Faculty of Medicine, University of Novi Sad, Serbia
[d] Institute for Forensic Medicine, Clinical Centre of Vojvodina, Faculty of Medicine, University of Novi Sad, Serbia

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ABSTRACT

Introduction: The relation of AAT phenotype and COPD still raises lots of controversy. In this study we aimed to investigate relation lung function characteristics, AAT serum level and COPD in smoking and non smoking population.
Patients and methods: This was a prospective non-randomized study in which we evaluated 45 patients with severe (stage IV) COPD. In all patients we determined AAT phenotype, serum AAT levels and lung function tests. We correlated findings in relation to the smoking status.
Results: All patients were MM type homozygotes. Serum AAT concentrations were within the reference values, amounting to 1.66 g/l in smokers and 1.80 g/l in nonsmokers. There was no significant correlation between serum AAT concentrations and lung function parameters. We have observed the higher mean values of ITGV, RV, TLC and RV/TLC in smokers and a statistically significant difference only in ITGV.
Conclusion: All of the investigated patients with severe COPD were MM type homozygotes with normal plasma level of AAT. There was no significant correlation between the phenotype and severity of COPD. We did not find significant relation of plasma AAT level and lung function impairment.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is the major morbidity and mortality cause in the world, representing a significant and increasing social and economic problem. COPD prevalence, morbidity and mortality significantly vary in different countries and even in different population groups of the same country, but they generally correlate with prevalence of smoking [1].

COPD is most commonly induced by smoking combined with genetic susceptibility. The best-described COPD-inducing genetic condition is alpha-antitrypsin deficiency. It is due to the SEPRIN 1 gene mutation. The SERPIN 1 gene's role is to give instructions for the production of alpha-antitrypsin protein, which is the serpin-type protein. The official name of this gene is "THE SERPIN PEPTIDASE INHIBITOR" (SERine Proteinase Inhibitor, alpha-1 antiproteinase, antitrypsin) [2,3].

α_1-antitrypsin (AAT, α_1-AT) is a typical secretory glycoprotein of 54 kDa molecular mass, which is translated on the granular endoplasmic reticulum, glycolized in the granular endoplasmic reticulum cistern's, translocated into the Golgi apparatus, and then secreted. The enzyme is primarily produced in the liver, but also in extrahepatic regions and cells (locally produced by macrophages and bronchial epithelial cells), including the tumorous cells, and then it is transported into the lungs by diffusion, via the blood [4,5].

AAT has a function of protecting the pulmonary parenchyma from the effects of neutrophil elastases (NE) — potent destructive proteases. In case of AAT deficiency, a gradual destruction of the pulmonary tissue occurs, resulting finally to chronic obstructive pulmonary disease (COPD), emphysema and early death. Alpha-antitrypsin belongs to the group of highly polymorphic proteins with over 123 variants determined at the level of proteins or genes [4,6].

Two most common mutations at the level of "deficiency" are PiZ (exon V, Glu^{342} *GAG*→Lys *AAG*), and PiS (exon III Glu^{264} *GAA*→Val *GTA*), while the rare ones include: I, MMalton, MPittsburg Mduarte, zero and others [7].

* Corresponding author at: Institute for Pulmonary Diseases of Vojvodina, Clinic for Pulmonary Oncology, Department for Interventional Pulmonology, Faculty of Medicine, University of Novi Sad, Institutski put 4, 21204 Sremska Kamenica, Serbia. Tel.: +381 21 480 5224, +381 64 26 36 187 (cell); fax: +381 21 480 5209.
E-mail addresses: bojanzaric@neobee.net, bojanzaric@gmail.com (B. Zarić).

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A few studies have established a correlation between the serum AAT levels and the genotype, with 10–15%, 51%, 83%, 97% and 100% serum AAT concentrations of ZZ, SZ, MZ, SS, MS and MM genotypes respectively [8].

The effect of the excessive AAT deficiency (ZZ genotype) on the lung function has already been recognized. The moderate AAT deficiency (MZ and SZ genotypes) as well as the MM genotype one, is less clear [9,10]. Some authors suggest the role of the AAT deficiency in COPD may have been overestimated.

The latest WHO guidelines and the Global Initiative for Chronic Obstructive Lung Disease (GOLD) of the American Thoracic Society and the European Respiratory Society recommend the screening campaign to detect the AAT deficiency in COPD patients. This strategy is of crucial importance in the countries with poor COPD diagnosing due to a low physicians' suspicion index [11,12]. It has to be emphasized that the overall medical application of this laboratory protocol requires cooperation of biochemists, molecular biologists and clinical physicians responsible for the treatment of the patients with a genetically conditioned alpha-antitrypsin deficiency [6]. As a few studies precisely defining the genotype frequency of the patients with pulmonary emphysema have been available here, this study is aimed at: Identifying the homo or heterozygote of the deficient gene allele for alpha-antitrypsin in COPD patients; Measuring serum alpha-antitrypsin concentrations in COPD patients; Establishing the correlation between the serum alpha-antitrypsin level and the genotype in COPD patients; Establishing the correlation between the serum alpha-antitrypsin and lung function disorders in COPD patients; and the correlation between the smoking habit and lung function disorders in COPD patients.

2. Patients and methods

The investigation was carried out at the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia, in 2010 and in the DNA Unit of the Forensic Medicine Institute of Novi Sad.

The material of the study included the venous blood samples from 45 unrelated subjects residing in Vojvodina, and hospitalized in the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, for pulmonary emphysema.

The venous blood samples were taken in tubes with an anticoagulant (EDTA), then making permanent blood stains made on FTA cards. The genomic DNA isolation from single samples will be performed by the FTA-card and Chelex-method combination [13,14,15].

Isolated nuclear DNAs were amplified by the method of real time polymerase chain reaction (Real Time PCR), placing the samples into the ABI Prism 7000 Sequence Detection System device (Applied Biosystems, Foster City, CA, USA).

The polymorphism stemming from the difference in a single nucleotide (the analysis of the allelic discrimination, between two allelic forms (S and Z)) was qualitatively detected using the End Point and the blood dissociation analyses. This method implies a simultaneous amplification and detection of PCR products.

The clinical diagnosis of pulmonary emphysema was established by functional tests of the respiratory system and the radiological, i.e. CT verification of the pulmonary parenchyma reduction and bullous lesions.

Serum alpha-antitrypsin levels were determined by the radial immunodiffusion method, using the human alpha$_1$-antitrypsin Nindarit™ Radial Immunodiffusion kit (Birmingham, B296AT UK).

Lung function tests were performed by the MasterScreen Body — VIASYS-Jeger appliance, measuring the following lung function parameters: intrathoracic gas volume (ITGV), residual volume (RV), total lung capacity (TLC) the residual volume vs. total lung capacity ratio (RV/TLC), vital capacity (VC), forced expiratory volume in the first second (FEV_1) the forced vital capacity in the first second vs. vital capacity ratio (FEV_1/VC), peak expiratory flow (PEF), forced expiratory flow at 50% vital capacity (FEF_{50}).

Blood gas analyses were performed by the AVL OMNI C instrument, measuring the following parameters: hemoglobin saturation with oxygen (SO_2), partial pressure of oxygen in the arterial blood (PO_2), partial pressure of carbon-dioxide in the arterial blood (PCO_2), blood pH level (pH), and bicarbonates (HCO_3).

Computerized tomography of the chest was performed by the VCT light speed 2007 device. The collected data were saved into the computer data base, and the statistical processing was done by the software JMP7, SAS Institute, Cary, NC. The continuous variables were presented as mean standard deviation values, while the categorical variables were presented as absolute values and in percents. The continuous variables were compared with the normal distribution using the Student t, or the Mann–Whitney *U* test in case of abnormal distribution, while the categorical variables were compared by the χ^2 test. To compare lung function parameters to the smoking history, we applied the Kruskal–Wallis test. The values $p \leq 0.05$ were evaluated as statistically significant.

3. Results

The examined group included 29 smokers at the mean age of 61.64 yrs and 16 non-smokers at the mean age of 66.00 yrs. All our patients with COPD were MM type homozygotes. Serum AAT concentrations were within the reference values, amounting to 1.66 g/l in smokers and 1.80 g/l in nonsmokers. In spite of the normal serum AAT concentrations, examined subjects had a severe COPD (stage IV COPD), as the accomplished mean FEV_1 was 30.5% and 35.20% in smokers and nonsmokers respectively, while FEV_1/FVC averaged 65.36% in smokers, and 75.29% in nonsmokers (SD 26.21). There was no significant correlation between serum AAT concentrations and lung function parameters. Lung function test results are expressed as the achieved percentile (%) of the predicted value, and PO_2 and PCO_2 in kPa and given in Table 1.

The measured mean ITGV values were 203.22% in smokers, and 173.13% in nonsmokers. The smokers' and nonsmokers' RV was 252.12% and 170.59% respectively. In smokers, TLC equaled 123.81, while in nonsmokers it was 113.16. The RV/TLC ratio was higher in smokers (191.05%) than in nonsmokers (170.59%).

In our investigation, we have observed the higher mean values of ITGV, RV, TLC and RV/TLC in smokers and a statistically significant

Table 1
Age, AAT level and lung function test results in smokers and non-smokers.

Parameter	Smokers (n = 29)		Non-smokers (n = 16)		p
	Mean (% achieved value)	SD	Mean (% achieved value)	SD	
Age	65.64	9.38	66.00	13.75	>0.05
AAT	1.66	0.47	1.80	0.43	>0.05
CRP	52.27	45.81	54.50	47.04	>0.05
ITGV[a]	203.22	45.56	173.13	51.78	<**0.05**
RV	252.12	67.99	220.10	82.92	<0.05
TLC	123.81	28.72	113.16	25.85	>0.05
RV%/TLC	191.05	27.31	170.59	11.68	>0.05
VC	45.58	9.48	47.61	18.92	>0.05
FEV_1	30.50	14.16	35.20	18.34	>0.05
FEV_1%/VC	65.36	23.89	75.29	26.21	>0.05
PEF	27.37	12.67	36.77	25.43	>0.05
MEF_{50}	13.90	15.27	17.46	14.77	>0.05
MEF_{75}	28	21.77	15	20.24	>0.05
Rtot	0.81	0.37	0.82	0.41	>0.05
Sr. Tot.	5.76	2.93	5.22	3.78	>0.05
PO_2 kPa	7.16	1.10	7.05	1.8	>0.05
PCO_2 kPa	5.73	1.24	6.33	1.8	>0.05
SO_2%	87.32	7.13	83.13	3.26	>0.05
HCO_3 mmol/l	27.54	4.71	28.01	4.89	>0.05
pH	7.42		7.40		>0.05

[a] Only statistically significant difference was found on ITGV value. Statistically significant p value is <0.05.