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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Association of alpha-1 antitrypsin level and lung function in patients with chronic obstructive pulmonary disease

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SUMMARY

Introduction/Objective Alpha-1 antitrypsin deficiency is a well established inherited risk factor for chronic obstructive pulmonary disease (COPD); however, alpha-1 antitrypsin level may result in different lung function reduction.

The aim of our study was to evaluate possible associations of alpha-1 antitrypsin level and lung function in COPD patients with different alpha-1 antitrypsin phenotypes.

Methods Serum alpha-1 antitrypsin concentration from patients (n = 1,167) with COPD, defined according to the GOLD criteria, were analyzed by nephelometry, and alpha-1 antitrypsin phenotype was determined by means of isoelectric-focusing.

Results In COPD patients without alpha-1 antitrypsin deficiency (MM), a significant negative association of lung function (FEV_1) with serum alpha-1 antitrypsin ($r = -0.511$; $p < 0.05$) and C-reactive protein (CRP) concentrations ($r = -0.583$; $p < 0.05$) was detected; moreover, the level of alpha-1 antitrypsin positively correlated with CRP concentration ($r = 0.667$; $p < 0.05$).

Conclusions In patients without alpha-1 antitrypsin deficiency, detected negative association of alpha-1 antitrypsin level with FEV_1 and positive association with the CRP level defined the importance of alpha-1 antitrypsin for lung function in COPD patients.

Keywords: chronic obstructive pulmonary disease; alpha-1 antitrypsin; lung function

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a prevalent and costly disease characterized by a progressive airflow limitation, related to an abnormal inflammatory response of the lung to long-term tobacco smoke exposure or inhalation of toxic gases [1]. Lung inflammation is further amplified by oxidative stress and proteolytic damage by proteinases [2, 3]. There is increasing data of systemic inflammation in patients with COPD [4–7]. Thus, the changes of inflammatory markers can be evaluated in the lungs and in serum affecting gas diffusion and lung function [3, 4, 5].

The best described inherited risk factor for COPD is alpha-1 antitrypsin (AAT) deficiency. Primary AAT function is to inhibit neutrophil elastase [6, 7, 8]. In severe AAT deficiency, anti-elastase protection in the lung interstitium and alveolar zone is decreased to about 15–20% of normal limits, similar to the decrease in serum levels [9–12]. The majority of AAT deficiency cases (96%) have a PI*ZZ phenotype. The remaining cases belong to PI*SZ, PI*MZ, and other especially rare deficiency phenotypes [9]. AAT is a rare disorder because it is underdiagnosed worldwide; more than 80% of AAT deficiency patients remain unrecognized [10].

The potential role of systemic inflammation in the pathogenesis of lung function decline in COPD patients with different AAT phenotypes has not yet been well established.

The aim of our study was to evaluate possible associations of AAT level and lung function parameters in patients with COPD with different AAT phenotypes.

METHODS

Sample sources and subject selection

The study content was approved by the Lithuanian Bioethics Committee. A total of 1,167 patients with COPD, who gave their informed consent, were included in the study at the Department of Pulmonology and Immunology, Medical Academy, LUHS.

Only patients who met the GOLD spirometric criteria for COPD: 1) ratio of post-bronchodilator forced expiratory volume in one second (FEV_1) to forced vital capacity (FVC) less than 0.7, and 2) FEV_1 less than 80% of the predicted

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value – were included in the study [1]. Patients with chronic or acute inflammation were excluded from the study. After an appropriate physical examination, data on the symptoms of the patient and the diagnosis of COPD was also collected. Smoking history was also calculated in pack-years as the product of tobacco use (in years) and the average number of cigarettes smoked per day / 20 (years × cig. per day / 20).

Sample collection and evaluation

Blood samples were taken in serum tubes, clotted at normal room temperature for 35–65 minutes and centrifuged for 15 minutes at 4,000 rpm. Then, the samples were frozen at -70°C for further analysis. The serum levels of AAT were determined by nephelometry using commercial kits (Dade Behring Marburg GmbH, Marburg, Germany) according to the manufacturer instructions. AAT phenotyping was carried out by means of isoelectric focusing (LKB Multiphor II and LKB Macrodrive 5 Constant Power Supply, Amersham Pharmacia Biotech, Piscataway, NJ, USA), as previously described [13]. The analysis of C-reactive protein (CRP) in serum was done using standard assays (IBL International GmbH, Hamburg, Germany).

Statistical analysis

Descriptive statistics were used to tabulate the primary cohort database. Quantitative variables were expressed as means with standard deviations. Differences of quantitative data were assessed by the Kruskal–Wallis H-test. Correlations between variables were determined by the Spearman correlation test. A p-value of less than 0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Demographic descriptions of studied 1,167 COPD patients are shown in Table 1**.** Eighty-two percent of the patients were current (57%) or former (25%) smokers of 22.1 ± 12.2 pack-years, and 18% never smoked.

Phenotype distribution was as follows: 1,076 (92.2%) PI*MM, 40 (3.4%) PI*MZ, 39 (3.3%) PI*MS, 1 (0.1%) PI*SS, 3 (0.3%) PI*SZ, and 8 (0.7%) PI*ZZ.

The mean AAT serum level (g/l) was of 1.58 ± 0.43. As expected, we found significant differences in AAT serum concentrations between groups ($p < 0.05$) (Figure 1)**.** The PI*ZZ group showed a markedly lower AAT blood level (0.40 ± 0.34) relative to the other AAT phenotype groups.

While analyzing lung function, the patients with AAT deficiency (PI*ZZ, PI*SZ, PI*SS) were grouped into one group. These individuals with severe AAT deficiency showed poorer spirometric FEV_1 (46 ± 20; $p < 0.05$) and FEV1/FVC (48 ± 16; $p < 0.05$) values than PI*MM, PI*MS, and PI*MZ patients (Table 2).

We found a statistically significant negative correlation between the AAT concentration and the FEV_1% predicted

Table 1. General data of study individuals

Variable	Values*
Age (years)	64 ± 12
Male/female	834 (71) / 333 (29)
Smoking status	
Smokers	660 (57)
Ex-smokers	294 (25)
Never-smokers	213 (18)

*Data are presented as n (%) or mean ± SD

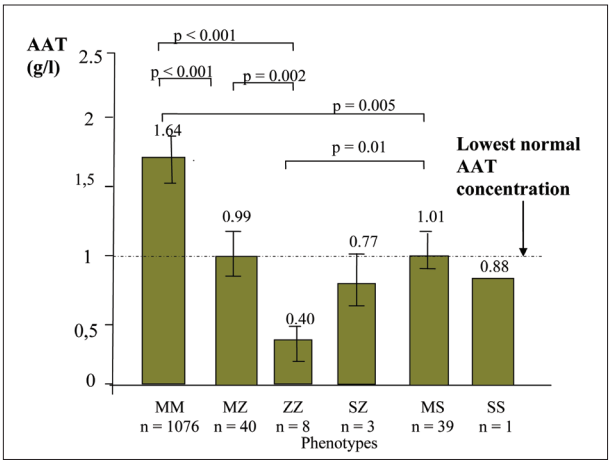

Figure 1. Serum alpha-1 antitrypsin (AAT) concentration in COPD patients with different AAT phenotypes

Table 2. Expresion of lung function and C-reactive protein (CRP) concentration in chronic obstructive pulmonary disease (COPD) patients with different alpha-1 antitrypsin phenotypes

Variable	MM n = 1,076	MS n = 39	MZ n = 40	SS, SZ, ZZ n = 12
FVC (% predicted normal)	74 ± 19	75 ± 15	73 ± 15	75 ± 17
FEV_1 (% predicted normal)	48 ± 17	51 ± 16	52 ± 18	46 ± 20
FEV_1/FVC (%)	54 ± 11	56 ± 11	57 ± 12	48 ± 16
CRP (mg/L)	9.6 ± 0.7	10.2 ± 0.8	11.3 ± 1.2	9.3 ± 1.5
COPD stage				
I	32 (3)	2 (5)	-	1 (8)
II	538 (50)	21 (54)	22 (55)	3 (25)
III	433 (40)	12 (31)	14 (35)	5 (42)
IV	73 (7)	4 (10)	4 (10)	3 (25)

Data are presented as n (%) or mean ± SD

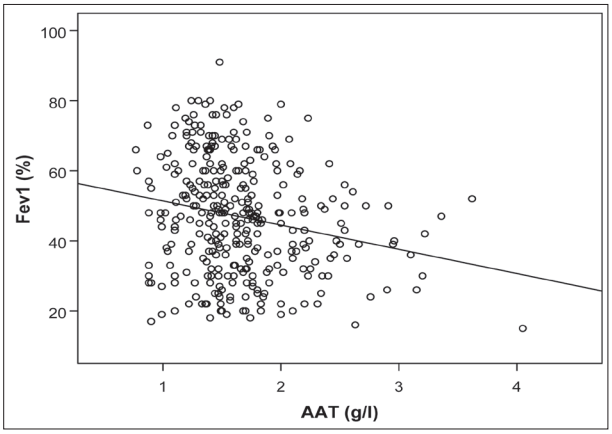

Figure 2. The correlation between the AAT concentration and FEV_1 in COPD patients without AAT deficiency (Spearman correlation coeficient)

Table 3. The correlation between the alpha-1 antitrypsin concentration and FEV_1 in chronic obstructive pulmonary disease patients without alpha-1 antitrypsin deficiency according to smoking status and sex

Patient group	n	r	Gender	n	r
Smokers	617	-0.511 ($p < 0.05$)	Male	415	-0.407 ($p < 0.05$)
			Female	202	-0.332 ($p < 0.05$)
Ex-smokers	249	-0.403 ($p < 0.05$)	Male	179	-0.398 ($p < 0.05$)
			Female	70	-0.178 ($p > 0.05$)
Never-smokers	210	-0.211 ($p > 0.05$)	Male	160	-0.166 ($p > 0.05$)
			Female	50	-0.152 ($p > 0.05$)

r – Spearman correlation coeficient

value in PI*MM phenotype ($r = -0.511$; $p < 0.05$) (Figure 2). While analyzing correlation between AAT concentration and FEV_1 in COPD patients according to smoking status and sex, we observed an inverse correlation in smokers and ex-smokers, but not in non-smokers. In males, this correlation was stronger than in females (Table3). Patients with elevated CRP were excluded from calculations. In addition, we detected that inverse correlations between CRP and FEV_1 have also been shown in COPD patients with the PI*MM phenotype ($r = -0.583$; $p < 0.05$). However, we didn't find such a correlation in COPD patients with AAT deficiency. In patients without the AAT deficiency, a significant positive association of blood AAT and CRP levels was detected ($r = 0.667$; $p < 0.05$).

DISCUSSION

The importance of the presented data is that circulating AAT inversely correlated with FEV_1 in COPD cases without the AAT deficiency. Such relationship had also been shown with healthy individuals [14, 15]. SAPALDIA project investigated associations of circulating AAT level with lung function in the general population and detected a negative correlation of serum AAT concentration with FEV_1 [14]. The amount of AAT that passively diffuses from the serum to the lung increases during an inflammation, which may be present in COPD [16]. This may indicate the increased need of AAT production to meet requirements of overcoming the release of various endogenous enzymes from inflammatory cells in the lungs, but its protective function may be overrun by the high level of secreted proteases [17]. However other studies have not found such a relationship between the serum AAT level and FEV_1% predicted value in COPD patients [18]. Possibly many other mechanisms might also be important for the pulmonary function, and not only for the inflammatory response.

Detected low AAT level in the PI*ZZ phenotype and the FEV_1 AAT ratio association may reflect a dual role of the AAT molecule as a pulmonary disease marker. The impact of AAT on pulmonary function seems to be a conclusion of context-dependent (i.e. AAT phenotype) and contrasting protective and proinflammatory effects in lung lining. On the one hand, elevated blood AAT level can show a beneficial shift in the antiprotease–protease balance, the center piece of the pathophysiological mechanism mediating the effect of most severe AAT deficiency on COPD. On the other hand, elevated blood AAT can also reflect low-grade inflammatory reaction in the lung [19, 20]. Significantly higher AAT concentration was even reported for AAT deficient (PI*ZZ) patients with COPD compared to PI*ZZ patients without COPD, further supporting the hypothesis that AAT concentration may also reflect an ongoing proinflammatory reaction [21]. Thus, our results support the hypothesis that the reduction of lung function may be a consequence of the presence of inflammatory stimuli.

Consistent with these findings, we could show a positive relationship between the AAT and CRP levels. High serum CRP concentrations in severe COPD individuals have been reported in other studies [5, 13, 14, 22]. Gan et al. [22] were the first to emphasize the importance of high CRP levels in COPD patients, showing the inflammatory process in even stable disease cases. Both CRP and AAT are acute-phase proteins. Several studies found CRP and AAT elevation in COPD patients, indicating that the inflammatory process is present in pathogenesis of the disease [5, 7, 21, 22, 23]. In addition, we find inverse correlations between CRP and FEV_1. Even in healthy individuals, an elevation of the CRP concentration over time was connected with a steeper FEV_1 decline [23, 24]. In these studies, FEV_1 was also inversely associated with blood CRP level. CRP reflects total systemic inflammation in many diseases and has been shown to upregulate the production of inflammatory cytokines [7]. The reasons for the inverse association between reduced lung function and systemic inflammation are not fully understood, but several mechanisms may be involved. Firstly, reduced pulmonary function may be responsible for the observed systemic inflammatory process. Inflammatory pulmonary epithelial cells have been shown to express small amounts of CRP and IL-6 [20, 25, 26]. Hence, the persistence of a systemic inflammatory process may result in damage to the airways, promoting the decline in FEV_1 in COPD patients. The data show that AAT has an immunomodulating capacity and acute increase in AAT level during various infectious and inflammatory states may enhance the magnitude of proinflammatory cells' reaction to endotoxic materials and subsequently accelerate the resolution of the inflammatory process.

CONCLUSION

We found that in patients without AAT deficiency, detected negative association of AAT level with FEV_1 and positive association with CRP level defined the importance of AAT as a biomarker of systemic inflammation for lung function in COPD. However, associations are complex and understanding the reactions of various mediators will require appropriately designed further studies.

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Повезаност нивоа алфа-1 антитрипсина и плућне функције код болесника са хроничном опструктивном болешћу плућа

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САЖЕТАК

Увод/Циљ Недостатак алфа-1 антитрипсина је добро познат наслеђен фактор ризика за хроничну опструктивну болест плућа (ХОБП). Међутим, низак ниво алфа-1 антитрипсина може узроковати различита смањења плућних функција.

Циљ нашег рада био је да се процени могућа повезаност нивоа алфа-1 антитрипсина и плућне функције код болесника с ХОБП са разним фенотипама алфа-1 антитрипсина.

Методе Концентрација серумског алфа-1 антитрипсина код болесника са ХОБП ($n = 1167$) у складу с критеријумима *GOLD* анализирана је коришћењем нефилометрије, а фенотип алфа-1 антитрипсина одређен је изолелекричним фокусирањем.

Резултати Код ХОБП болесника без недостатка алфа-1 антитрипсина (ММ) пронађена је значајна негативна повезаност плућне функције (FEV_1) са серумом алфа-1 антитрипсина (r =- 0,511, $p < 0,05$) и концентрацијом ЦРП (r = -0,583, $p < 0,05$); осим тога, ниво алфа-1 био је у позитивној повезаности са концентрацијом ЦРП ($r = 0,667$, $p < 0,05$).

Закључак Код ХОБП болесника без недостатка алфа-1 антитрипсина пронађена је значајна негативна повезаност са FEV_1 и позитивна повезаност са нивоом ЦРП доказала је значај алфа-1 антитрипсина као показатеља системске инфламације.

Кључне речи: хронична опструктивна болест плућа; алфа-1 антитрипсин; функција плућа