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THE LEVEL OF ALPHA-1 ANTITRYPSIN IN INHERITED LUNG DISEASES

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ABSTRACT

Alpha1-antitrypsin deficiency is underdiagnosed condition in patients with chronic obstructive pulmonary disease. Serum alpha-1 antitrypsin concentration from patients with chronic obstructive pulmonary disease, defined according to the GOLD criteria, were analysed by nephelometry, alpha-1 antitrypsin genotype was determined by means of isoelectric-focusing. Calculated sensitivity of quantitative alpha-1 antitrypsin measurement by nephelometry for heterozygous PI*Z allele is 45% and for homozygous ZZ genotype 88%. Specificity of quantitative alpha-1 antitrypsin deficiency determining analysis is 99%.A case detection programme of alpha-1 antitrypsin deficiency in patients with chronic obstructive pulmonary disease using quantitative methods is specific, but due to limited sensitivity should be used only in screening programs.

Key words: Chronic obstructive pulmonary disease, alpha-1 antitrypsin, sensitivity.

INTRODUCTION

Alpha1-antitrypsin (AAT) is characterised by abnormally reduced serum AAT concentration, which, in homozygote form, carries a high risk of developing early pulmonary emphysema or chronic obstructive pulmonary disease (COPD) [1]. AAT is a circulating serine proteinase inhibitor (PI) secreted by the liver, which permeates most body tissues where it acts as an inhibitor of a range of proteolytic enzymes. The most of the pathology related to AAT deficiency is linked to the PI*Z allele, and in clinical practice, 96% of AAT deficiency patients have a homozygous genotype ZZ [2]. Less severe AAT deficiency is caused by heterozygous genotype: -Z. AAT deficiency is an under-diagnosed condition worldwide [2]. Recent guidelines from both the World Health Organization and the American Thoracic Society/European Respiratory Society [2] recommend the establishment of screening programs for the detection of AAT deficiency in patients with COPD [3], because the detection of coexisting AAT deficiency could lead to family screening, appropriate management (including lifestyle changes such as quitting smoking and replacement therapy in selected cases), and specific counselling for these patients and families [4]. AAT deficiency can be suspected by quantitative serum analysis, however only detection of gene mutation confirms exact diagnosis. The aim of our study was to evaluate sensitivity of quantitative method, that is usually used for screening of AAT deficiency.

METHODS

The study design was approved by the Regional Ethics Committee. 1167 patients with COPD were offered to participate in the study. Descriptive statistics were used to tabulate the primary cohort database. Quantitative variables were expressed as means with standard deviations (SD). Differences of quantitative data were assessed Kruskal-Wallis *H* test. A *P* value of less than 0.05 was considered significant. Statistical analysis was performed with the SPSS 14.0 program. Statistical sensitivity and specificity were calculated by binary classification test:

$$\text{sensitivity} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}$$

$$\text{specificity} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}$$

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RESULTS

The genotypes and the corresponding AAT concentration of 1167 COPD patients are shown in Table 1.

Table 1. Demographic characteristics, spirometric values , AAT genotypes and serum concentrations from 1167 COPD patients

AAT genotypes	N	Sex % M	Age (SD)	FEV1% pred.	AAT serum concentration
NoZ (- -)	1119	84	66 (10.4)	46 (15.8)	164.7 (39.8)
Heterozygous Z (-Z)	40	65	67 (11.2)	51.5 (14.5)	99 (14)
Homozygous Z (ZZ)	8	62	54 (11.3)	38.5 (18.1)	43(12.4)
TOTAL	1167	82	62 (10.3)	46.5 (15.9)	158 (43.6)

Data are presented as mean or %, unless otherwise stated.
N: Number. P
AAT: Alpha1-antitrypsin. %: percent.
SD: Standard deviation. M: Males.
FEV1: forced expiratory volume in one second expressed in percent of the predicted normal value.
AAT serum concentrations measured by nephelometry, values expressed in mg/dL.

As expected, significant differences in AAT serum concentrations between groups were found (P=0.01). The ZZ group showed a significant lower AAT serum concentration (43mg/dL). Nearly eighty- three percent of the patients were current (57.6%) or former (25.2%) smokers of 22.1 ±12.2 pack-years, and 17.2% never smokers..

All 1167 samples were processed both for the quantification of AAT and determination of PI*Z deficiency allele. Concentrations above the cut-off point established as normal were detected in 1134 samples (97.2%) and 33 samples (2.8%) presented low concentration. Among the individuals with normal concentrations, the PI*Z allele was not detected in 1111 (98.0%) and was detected in 23 (2%) patients.

Calculated sensitivity of quantitative AAT measurement by nephelometry for heterozygous PI*Z allele was 45% and for homozygous ZZ genotype - 88%. Specificity of quantitative AAT deficiency determining method was 99%.

DISCUSSION

Although the diagnosis of AAT deficiency is relatively simple, population studies have indicated that this disease is underdiagnosed and a delay in diagnosis is very common up till now [7]. The quantifying of AAT concentration is very important for identifying individuals with congenital AAT deficiency in screening purposes. However our study showed, that quantitative method's sensitivity for heterozygous PI*Z mutation is only 45% and for homozygous ZZ genotype 88%. The most striking finding was that even a severe homozygous ZZ deficiency was found in one subject with normal AAT concentration. We speculate that it could be because of bronchial damage due to terminal stage of disease. This COPD patient had very low FEV_1 value (15% predicted) although general inflammatory markers were not elevated (CRP 3mg/l). It shows that the presence of false negative results didn't allow all samples with normal concentrations to be qualified as nondeficient. FEV1 also was inversely associated with CRP concentration. CRP reflects total systemic burden of inflammation in several disorders and has been shown to upregulate the production of proinflammatory cytokines. The reasons for the inverse association between systemic inflammation and reduced pulmonary function are not fully clear, but several mechanisms may be involved. Firstly, reduced lung function may be responsible for the observed systemic inflammation. Inflammatory lung or pulmonary epithelial cells, have been shown to express CRP and IL-6. IL-6 may reach the bloodstream, stimulating the production of CRP and other inflammatory mediators by the liver, sequentially activating pulmonary inflammatory cells during transit through the pulmonary circulation. An alternative mechanism— reverse causation—cannot be excluded: high levels of cytokines and acute-phase reactants in the peripheral circulation may be a cause rather than a consequence of poor lung function. There is increasing evidence that cytokines play a major role linked to the activation and adhesion of inflammatory cells to the pulmonary capillary endothelium, leading to changes in endothelial function and increases in pulmonary vascular filtration. Besides it is observed that COPD may influence even venous circulation. Thus persistence of systemic inflammation, may result in damage to the airways, accelerating decline in FEV1 of COPD patients. Another mechanism leading to a protease-antiprotease imbalance in the lung during PI*ZZ antitrypsin deficiency, is that an abnormal Z antitrypsin polymerizing in the lung, acts as a neutrophil chemo-attractant, leading recruitment of neutrophils to the lung. It was recently shown, that AAT regulates many physiological and pathological processes, which may significantly influence the disease process including cell mediated immunity, apoptosis, tumor cell growth and many others. AAT has been shown to regulate even a receptor for lypopolysaccharides - CD14 molecules expression in human monocytes in vitro.

Important marker, that have been analyzed in our study is TNF-α. This inflammatory cytokine is important in COPD pathogenesis and in the mechanism for the function of AAT . In vitro studies have demonstrated inhibition of

TNF-α, production by AAT [36]. It is observed that TNFα levels may be elevated in the sputum, bronchial biopsies and circulation of COPD patients . Other investigators analysing TNFα level in COPD patients didn‘t find any association with the severity of disease . Explanation for that could be, that these cytokines mainly acts in peripheral lung tissues and their level differences could be detected in induced sputum but not always in systemic circulation. Thus, in our study sTNFR-1 had positive correlations with inflamatory markers: AAT and CRP. There was also positive association between sTNFR-1 and sTNFR-2. These soluble receptors, which inhibit the inflammatory effect of TNFα are expresed and released from many different cells, enabeling even elevation of concentration in systemic circulation, were they can be detected .

In heterozygous state quantitative test may not allow detection of some individuals possibly due to pathophysiological inflammatory processes. Even smoking in COPD patients may be associated with higher AAT and CRP production in the liver of COPD patients and mechanisms connected with systemic inflammation which continues even after smoking cessation. Even in healthy individuals, positive associations between active smoking and AAT levels have been reported before [8]. The quantity of AAT that diffuses passively from the blood to the lung increases during an inflammatory process, which take place in COPD [9]. This may indicate increased requirement of AAT to meet the needs of overcoming the release of various enzymes from neutrophilic cells in the lungs, but its protective function may be overrun by the high concentration of proteases [10]. Increase AAT level in smokers and ex-smokers reflect the dual role of AAT as a respiratory disease biomarker. The net impact of AAT on lung function seems to be a result of context-dependent (i.e. AAT genotype) and contrasting protective and inflammatory effects in respiratory tract. On the one hand, elevated serum AAT can reflect a beneficial shift in the protease-antiprotease balance, the centre piece of the pathophysiological pathway mediating the effect of severe congenital AAT deficiency on COPD. On the other hand, elevated serum AAT can also reflect low-grade inflammatory processes in the lung [11] it is hypothesized COPD risk factor.

In European countries AAT deficiency detection programs have been carried out by using different methodologies. Case control studies demonstrated an increase in the prevalence of PI*Z heterozygotes in patients with COPD compared with the control group when using genotyping methods [12-15].

CONCLUSIONS

The results of the present study support the general concept of targeted screening for AAT deficiency with adequate laboratory methods in European countries with PI*Z high frequency and large population of COPD patients with highest diagnostic value - AAT genotyping. A case detection programme of alpha-1 antitrypsin deficiency in patients with chronic obstructive pulmonary disease using quantitative methods could be used only in screening programs and exact diagnosis must be confirmed by determining AAT genotype.

In conclusion, when desining a case detection programme, both the protocol of sample processing and the inclusion criteria for the candidates should be taken to the account, since both factors have a decisive influence on the performance of the programme.

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