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ORIGINAL ARTICLE

Phenotypes and serum level of alpha-1-antitrypsin in lung cancer

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Summary

Purpose: *Alpha-1-antitrypsin (AAT) as the major circulating inhibitor of proteases has important role in protease-antiprotease homeostasis. Recent studies have confirmed its anti-apoptotic role. AAT is a highly polymorphic protein. Individuals with normal variants have normal serum levels and functional activity of ATT. However, individuals with hereditary AAT deficiency (AATD) have low circulating levels of AAT. Severe AATD was identified as genetic risk factor for early onset of pulmonary emphysema. Association between AAT phenotypes and lung cancer (LC) is not clear, and different studies show contradictory results. The aim of this case-control study was to investigate phenotypes and serum level of AAT in LC.*

Methods: *The study group included 147 patients with LC, classified as small cell lung cancer (SCLC, n=42) and non-small cell lung cancer (NSCLC, n=105). The control group consisted of 273 healthy blood donors. AAT phenotyping was performed by isoelectric-focusing and AAT concentration was measured using nephelometry.*

Results: *There were no differences in the frequencies of AAT phenotypes and alleles between the control group and LC patients, as well as between NSCLC and SCLC groups. An elevated level of AAT was obtained in LC patients. PiMZ and PiMS phenotypes in LC patients were not deficient in the classical sense. AAT levels were 90 and 134%, respectively, when compared to PiMM phenotype in the control group.*

Conclusion: *Our findings revealed that moderate deficiency of AAT is not risk factor for LC development. Although polymorphism of AAT was not associated with risk of LC, further research of this antiprotease and antiapoptotic protein could clarify its role in carcinogenesis, given its high concentration in LC patients, even in AATD patients.*

Key words: alpha-1-antitrypsin, lung cancer, polymorphism, serum level

Introduction

AAT is the archetype member of the superfamily of structurally related proteins called SERPINS (**Ser**ine **P**roteinase **In**hibitor**s**). AAT is the major circulating inhibitor of many proteases, which has an important role in the maintenance of protease-antiprotease homeostasis and prevention of proteolytic tissue damage and cell migration. The target proteases of AAT are from the azurophilic granules of polymorphonuclear neutrophils which contain 3 serine proteinases: elastase, cathepsin G, and proteinase 3. The main physiological role of AAT is to inhibit neutrophil elastase (NE) in the lower respiratory tract to protect the connective tissue from NE released from triggered neutrophils. AAT is an acute-phase glycoprotein (52 kD), synthesized in hepatocytes and subsequently secreted into the plasma. It is also produced in smaller quantities by alveolar macrophages, circulating monocytes and in lung-derived epithelial cells, as well as by tumor cells. The SERPINA1 gene or the Pi (**P**roteinase **I**nhibitor) locus is on chromosome 14q32.1 and shows an autosomal codominant pattern of inheritance. Its product is a highly polymorphic protein with over 100 naturally occurring variants. The wild type, and the most prevalent allele is PiM with normal serum level, functional activity to inhibit NE and not connected with any disease. However, variants named deficient, dysfunctional and null are clinically significant. Deficient variants are associated with AATD in plasma, and connected with liver disease in neonates, and early-onset emphysema in adults. The most common deficiency alleles are PiZ and PiS, and their prevalence varies among populations. The estimated gene frequencies (per 1,000) of Z and S alleles in Eu-

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ropean populations are 2-24 and 1-9, respectively, while in USA they are 1-2 and 2-4, respectively [1]. The PiZZ homozygotes have only 15% of the serum level compared to the normal PiMM phenotypes, while PiMZ heterozygotes have about 50%. Carriers of S allele, PiSS, PiSZ and PiMS have mean AAT levels 52, 32 and 75% according to the PiMM, respectively.

In addition to its antiprotease role, there is considerable evidence that AAT exhibits other biological activities, such as antiapoptotic and antiinflammatory [2,3].

In recent decades, efforts are underway to identify the target polymorphisms that serve as biomarkers of genetic susceptibility to LC for specific populations. The role of AAT in the development of cancer is still unknown. Several studies demonstrated increased expression of AAT in malignant cells, and proposed utilization of SERPINA1 as a marker of malignancy [4,5]. Higashtyama et al. [6] showed that AAT expression status in lung adenocarcinoma cells may represent a prognostic biological marker of tumor growth. Boskovic et al. [7] observed that production of AAT by tumor cells is low compared with hepatocytes. These authors concluded that the AAT originated from malignant cells protects tumor tissues against potentially destructive proteases released by surrounding inflammatory cells. All these observations suggested that the tumor cell-derived AAT may play a significant role in tumor biology.

The aim of this case-control study was to investigate phenotypes and the serum level of AAT in LC. We analysed patients with SCLC and NSCLC. These two types of LC differ significantly in terms of incidence, aggressiveness and outcome. SCLC comprises about 20% of all LC cases and is the most aggressive lung tumor with poor clinical outcome. NSCLC is the most common form of LC representing 80-85% of all cases, less aggressive than SCLC and displays a slightly more favorable clinical outcome which depends mainly on the stage of disease at the time of diagnosis.

Methods

Study subjects

This study was performed in patients with primary LC admitted to the Zvezdara University Medical Center, Belgrade, Serbia. The protocol was approved by the local research ethics committee and oral informed consent was obtained from all study participants.

This study included 147 patients diagnosed with primary LC within one year prior to study inclusion. Patients were histologically classified as having SCLC (n=42) and NSCLC (n=105). Only 9% LC patients had a positive history of chronic obstructive pulmonary disease (COPD). For each patient, information regarding smoking status was obtained. The control group included 273 healthy blood donors. Out of this number, in 84 healthy subjects the serum concentration of AAT was determined.

Methods

Five ml of venous blood were taken from all patients and controls. Sera were separated by centrifugation at 3000 rpm for 15 min and stored at -80° C until analysis. Pi phenotype was determined using isoelectric focusing, pH range 4.2-4.9 (Pharmacia LKB Biotechnology, Uppsala, Sweden). Serum concentration of AAT was performed by nephelometric endpoint detection (Turbox™ α_1 Antitrypsin Assay. Orion Diagnostica. Espoo, Finland).

Statistical analysis

The differences in the frequencies of AAT phenotypes and alleles between patients and controls were analysed using the x^2 test (2×2 contingency table). Fisher's exact test was used when n<5. The analysis of variance was used for comparison of AAT serum concentration in LC patients and the control group. *Post hoc* comparisons were determined by the least significant difference (LSD) if the *F*-value for the parameters was significant. P-values of < 0.05 were considered as significant. For statistical analysis the STATISTICA 7.0® software was used.

Results

The frequencies of AAT phenotypes in LC patients (all LC patients, NSCLC patients, SCLC patients) and in the control subjects are shown in Table 1. There were no statistically significant differences between the control group and LC patient group, or between NSCLC and SCLC groups.

The cumulative frequency of moderate AATD phenotypes, PiMZ and PiMS was also analysed. Again, there was no difference in the frequencies of moderate AATD phenotypes between LC patients (all, NSCLC and SCLC) and the control group, as well as between NSCLC and SCLC.

The obtained serum concentrations of AAT in 147 LC patients and 84 healthy individuals are shown in Figure 1. Since only one PiMP phenotype was detected in the controls, it has been excluded from analysis.

The data shown in Figure 1 indicate that: (1) the

Table 1. Comparison of alpha-1-antitrypsin phenotype frequencies between LC patients and control

	All LC (n=147)	*NSCLC (n=105)*	*SCLC (n=42)*	*Controls (n=273)*	*p-value* All LC/NSCLC/SCLC*	*p-value***
Phenotypes						
MM	0.9184	0.9143	0.9286	0.9451	0.287/0.271/0.233	0.251
MZ	0.0408	0.0476	0.0238	0.0330	0.679/0.179/0.371	0.321
MS	0.0272	0.0286	0.0238	0.0183	0.221/0.237/0.395	0.421
MZ+MS	0.068	0.0762	0.0476	0.0513	0.497/0.858/0.295	0.253
MP	0.0136	0.0095	0.0238	0.0037	0.238/0.402/0.231	0.411
Alleles						
M	0.9592	0.9571	0.9643	0.9725	0.295/0.279/0.229	0.251
Z	0.0204	0.0238	0.0119	0.0165	0.682/0.178/0.369	0.319
S	0.0136	0.0143	0.0119	0.0092	0.223/0.236/0.393	0.419
Z+S	0.0340	0.0381	0.0238	0.0257	0.483/0.833/0.251	0.251
P	0.0068	0.0048	0.0119	0.0018	0.238/0.401/0.231	0.409

*LC vs. control, ** NSCLC vs. SCLC, LC: lung cancer, NSCLC: non small cell lung cancer, SCLC: small cell lung cancer. For other abbreviations see text

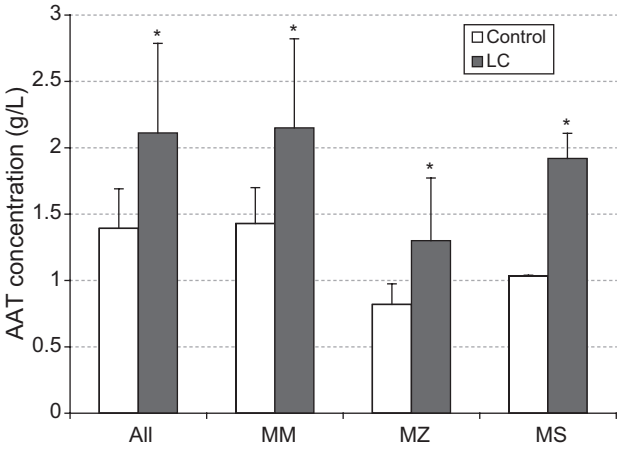

Figure 1. Histograms of serum concentration of AAT in control and lung cancer (LC) patient groups (means ± SD). *Statistical difference between LC patient and control groups. P-value for all phenotypes 0.00; for PiMM 0.00; for PiMZ 0.05; for PiMS 0.01.

Table 2. Serum concentration of AAT in 147 LC patients

LC patients	*N*	*AAT serum levels mean ± SD (g/L)*	*p-value*
Gender			
Males	57	2.11 ± 0.668	0.789
Females	90	2.08 ± 0.723	
Age (years)			
< 60	93	2.11 ± 0.756	0.918
≥ 60	54	2.12 ± 0.517	
Smoking status			
Smokers	132	2.13 ± 0.681	0.316
Non-smokers	15	1.93 ± 0.624	
Histological LC type			
NSCLC	105	2.16 ± 0.695	0.145
SCLC	42	1.98 ± 0.616	
AAT phenotypes			
MM	135	2.15 ± 0.671	
MP	2	2.47 ± 0.356	0.019
MS	4	1.92 ± 0.189	
MZ	6	1.30 ± 0.472[a,b]	

For abbreviations see footnote of Table 1
[a]compared with PiMM (p=0.002), [b]compared with PiMP (p=0.032)

serum concentrations of AAT in all LC patients, as well in LC patients with different AAT phenotypes were significantly higher than in the control group; (2) increasing levels of AAT in all LC patients, as well as in PiMM, PiMZ and PiMS LC patients compared to the controls were 51, 50, 58 and 85%, respectively; (3) the levels of healthy individuals with moderate AATD phenotypes (PiMZ and PiMS) were 57 and 72% compared to the normal PiMM phenotype, and in the LC patient group were similar: PiMZ and PiMS levels were 60 and 89% compared to the normal PiMM phenotype; (4) in LC patients with moderate AATD phenotypes, PiMZ and PiMS levels of AAT almost reached and even exceeded the level of healthy PiMM phenotype (90 and 134%, respectively).

Furthermore, we examined the concentration of AAT in the group of LC patients (Table 2). There was no difference in the concentration of AAT by gender, age, smoking status and histological type of LC cancer. However, patients with PiMZ phenotype had a significantly lower concentration of AAT compared to the PiMM (p=0.002), as well as in relation to the PiMP phenotype (p=0.032).

Discussion

Associations of AATD with COPD, as well as of COPD with LC are well known. Both diseases are related to cigarette smoking. It can be assumed that COPD caused by AATD could be a risk factor for LC, but this has not been sufficiently explored. Up until now, a small number of studies focused in the role of AAT in carcino-

genesis, but only a few in LC. It is considered that due to the physiological role to protect the lower respiratory tract from NE, AATD could create an enabling environment for local development of cancer in the lung. On the other hand, the proven antiapoptotic role of AAT [2] could be significant in carcinogenesis over this issue although its role still remains largely unclear.

In our previous study [8] we observed that moderate AATD phenotypes had a higher risk of developing squamous cell LC than those with non-AATD. However, this difference was not found between all LC patients and controls. Also, our current research has not demonstrated any association between AAT phenotypes and LC, both in all LC patients and in LC histological subtypes. Literature data regarding association of hereditary AATD and LC gave conflicting results [9-15], that could be explained by a number of causes. Generally, AATD phenotypes are relatively rare, and frequency of AATD homozygotes is much lower compared to AATD heterozygotes [1]. Besides, other causes of disagreement among studies could be due to the differences in the frequency of AATD variants in various populations [16].

As mentioned above, severe AATD represents a proven risk factor for COPD in adults. Therefore, it is logical to expect higher incidence of AATD phenotypes in LC patients who had history of COPD. However, in our study, only 9% of LC patients had history of COPD. This fact could explain the lack of higher frequency of AATD phenotypes in our LC patients group. Our previous study [17] revealed that the cumulative effect of non-MM variants of AAT (PiMZ, PiMS, PiMP) and intermediate or high activity of NE could represent a risk factor for aggressive primary LC associated with extrathoracic metastases. Similar results were obtained in the study by Yang et al. [14], which revealed that AATD genotypes and/or an excess of NE are significantly associated with LC risk. According to these facts, it can be assumed that both polymorphisms of AAT and NE may represent an additive risk for LC.

Our results showed significantly increased levels of AAT in LC patients compared with healthy subjects (Figure 1), in concordance with many studies [18-23]. Moreover, Varela et al. [24] suggested that plasma level of AAT can be an acceptable cancer marker that discriminates cancer from chronic non-cancerous diseases and complete clinical remission from relapses. Zelvyte et al. [20] revealed that AAT levels may provide measures of cancer progression in individual patients. Although it is known that AAT, as an acute phase reactant, is elevated in various malignancies, the clinical significance of its increased expression in cancer is still unclear.

It is interesting that we showed that increasing serum levels of AAT in normal PiMM and in deficient PiMZ LC patients in comparison with controls are similar. However, PiMS variant showed higher increase. We have also observed that PiMZ and PiMS heterozygotes among LC patients were not deficient in the classical sense, given that the AAT levels were 90 and 134%, respectively, compared with PiMM in healthy controls. It was also shown that decrease in AAT serum concentration in subjects with PiMZ and PiMS phenotypes in comparison with subjects with normal PiMM was similar in the control and LC patient group.

These results, and no or weak association between AATD polymorphism and LC suggest that lung carcinogenesis is not solely caused by an imbalance of proteases-antiproteases, as assumed in some studies [13-15].

In addition, the level of AAT in LC patients did not differ in relation to gender, age, smoking status and histological LC types. On the other hand, we observed significantly decreased level of AAT in PiMZ LC patients in comparison with PiMM and PiMP patients, but as already mentioned, this level was almost normal.

In summary, our findings revealed that moderate deficiency of AAT is not risk factor for LC development. Although polymorphism of AAT was not associated with risk of LC, further research of this antiprotease and antiapoptotic protein could clarify its role in carcinogenesis, given its high concentration in LC patients, even in AATD patients.

Acknowledgements

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