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Association of Moderate Alpha-1 Antitrypsin Deficiency with Lung Cancer in the Serbian Population

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Background. Alpha-1 antitrypsin (AAT) is an important serine protease inhibitor in human plasma. Its major physiological role is to inhibit neutrophil elastase (NE) in the lower respiratory tract and protect lung tissue from destruction. Recent studies indicated an etiological role of NE in lung cancer development. The aim of this study was to investigate the association of alpha-1 antitrypsin deficiency (AATD) with lung cancer in patients with four different histological types of cancer: squamous cell carcinoma, adenocarcinomas, large cell carcinoma and small cell carcinoma.

Methods. Phenotyping was carried out by isoelectric focusing (pH 4.2–4.9). We compared the frequency of AATD phenotypes in 186 lung cancer patients with the value obtained in our previous study in a healthy Serbian population (3.7%) using the Fisher exact test.

Results. Allele frequencies in patients were Pi*M 0.9677, Pi*Z 0.0215, Pi*S 0.0081 and Pi*other rare 0.0027. Eleven of the 186 lung cancer patients (5.9%) were AATD heterozygotes with moderate deficiencies (PiMZ and PiMS). When this value was compared with AATD heterozygote frequency obtained in the healthy individuals (3.7%), the difference was close to the level of significance ($p = 0.055$). However, individuals with AATD phenotypes had a higher risk of developing squamous cell lung cancer then those with non-deficient AAT variants (OR = 4.51, 95% CI = 1.66–12.29).

Conclusions. Our findings provide evidence of an association between AAT phenotypes with moderate deficiency and squamous cell lung cancer.

Key Words: Serine protease inhibitors, Alpha-1 antitrypsin deficiency, Lung cancer, Neutrophil elastase.

Introduction

Alpha-1 antitrypsin (AAT) is a highly polymorphic plasma glycoprotein (53 kD) mostly secreted by hepatocytes and, to a lesser extent, by lung epithelial cells and phagocytes. It belongs to the superfamily of structurally related proteins called SERPINs (SER*ine* P*roteinase* IN*hibitors*) that control many physiological reactions. Although AAT inhibits a variety of serine proteases, its major physiological role is to inhibit neutrophil elastase (NE) in the lower respiratory tract and protect the connective tissue from NE released from triggered neutrophils.

The AAT protein is encoded by the protease inhibitor (PI) locus located on human chromosome 14q32.1, which consists of seven exons and six introns spanning 12 kb. The protein includes 394 amino acids with the active site of the enzyme inhibitor at Met^{358}. Polymorphism of AAT has been extensively studied in different populations. More than 90 codominant alleles at the protease inhibitor locus have been identified. Differences in migration of protein variants on gel electrophoresis have been used to identify the PI phenotype

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system (1). The most common alleles are the M variants with allele frequencies >0.95 and with normal AAT concentration and functional activity. The common variants that lead to a plasma deficiency of AAT are Z and S and uncommon deficient alleles include I, M_{Malton}, $M_{Pittsburgh}$, S_{iiyama}, null and several other very rare alleles. Deficiency of AAT in Pi ZZ homozygote (10−15% of normal plasma level) can result in early-onset chronic obstructive pulmonary diseases (COPD) including emphysema and chronic bronchitis as well as liver disease, expressed as neonatal cholestasis that may progress to juvenile cirrhosis and slowly progressive liver disease in the adult.

However, the risk of heterozygote carriers of Z and S alleles (including Pi MZ, Pi SS, and Pi MS individuals) with moderate plasma AAT concentrations (50, 52 and 75%, respectively) for developing COPD remains controversial (2).

In addition, deficiency of AAT was investigated in different malignant diseases. Although recent studies have suggested that AATD is associated with increased risk of different types of cancer: liver cancer, bladder cancer, colorectal cancer, gallbladder adenocarcinoma and malignant lymphoma, the exact role of AATD variants as risk factors in lung cancer development is still unknown. The first well-documented study of Yang et al. (3) showed that individuals who carry an AATD allele may have increased risk for developing lung cancer. In the larger case-control study (4), the same authors found that the excess of neutrophil elastase or the deficiency of AAT increases lung cancer risk. They hypothesized that an imbalance between AAT and NE, smoking, and COPD were significant risk factors for lung cancer.

The aim of this study was to explore the association of deficiency of AAT and lung cancer in the Serbian population. The occurrence of AATD phenotypes in lung cancer patients was compared with the corresponding value in healthy subjects. Because lung cancer is a very heterogeneous disease, we also examined the AATD variants rate according to the histological type of tumor. We also investigated AATD variants in lung cancer patients by smoking status.

Materials and Methods

Study Subjects

The patients group consisted of 186 patients with primary lung cancer, admitted to the Institute of Lung Disease and TB, University Clinical Centre of Serbia, Belgrade during a 6-month period. There were 158 males and 28 females, aged 27−75 years (mean ± SD: 55.6 ± 9.7). The patients were recruited without any gender, age, histological or stage restrictions, but subjects with a prior history of malignant diseases were not included in the study. The majority of the patients were smokers (94%). Lung cancer was confirmed histologically according to the World Health Organization Histological Typing of Lung Tumours (5), and four types of lung cancer were identified: adenocarcinoma (AC, $n = 49$), small cell lung cancer (SCLC, $n = 53$), squamous cell carcinoma (SqCC, $n = 33$) and large cell carcinoma (LCC, $n = 51$). Results obtained in our previous investigation of AAT polymorphism in 1060 healthy Serbian blood donors (6) were used in this study for comparison, instead of a control group. The reported gene frequencies in the Serbian healthy population were 0.013 for the Pi^Z allele and 0.0070 for the Pi^S allele. The frequency of Z and S carriers (MZ and MS heterozygotes) was 3.7%.

Laboratory Methods

PI phenotyping was carried out by isoelectric focusing (pH range: 4.2−4.9) according to the method by Kishimoto et al. (7). The 0.2-mm thin polyacrylamide gels were casted by using Pharmacia LKB Ultromould Gel Casting Unit (Pharmacia, Uppsala, Sweden). Isoelectric focusing was performed with LKB 2117 Multiphor system. Serum samples were pretreated by dithioerythritol. Isoelectrofocusing is accepted as the ''gold standard'' for diagnosing AATD (8).

AAT antigen concentration was measured by an immunoturbidimetric method, using TURBOX (Orion Diagnostica, Espoo, Finland) reagents. AAT functional activity was assessed by measuring trypsin inhibitory capacity (TIC) using the method of Dietz et al. (9), and the specific inhibitory activity (SIA) was calculated as the trypsin-inhibitory capacity/AAT antigen concentration ratio.

Statistical Analysis

Fisher exact test was used to compare the frequency of AATD phenotypes among lung cancer patients with the frequency obtained in the healthy individuals; p values <0.05 were considered as significant. The risk for lung cancer development among individuals with AATD phenotypes was estimated by calculating odds ratio (OR) with 95% confidence interval. All statistical manipulations were done using internet statistical package: Simple Interactive Statistical Analysis (SISA).

Results

Demographic characteristics and laboratory results of the patients and healthy controls included in the study are shown in Table 1. A significantly higher prevalence of males and smokers, as well as higher AAT concentrations and erythrocyte sedimentation rates, were found among the patients as compared with the healthy blood donors.

In lung cancer patients, 8/11 AATD phenotypes carried the Z allele (PiMZ phenotype), whereas three were S allele carriers (PiMS phenotype). PiZZ homozygotes were not identified in the lung cancer patient group.