Are Serum Alpha-1-Antitrypsin and Alpha-2-Macroglobulin Levels Related with Tumor Histology or Stage of the Lung Cancer?

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ABSTRACT

In certain types of cancer, increased serum protease inhibitor levels were identified and clues showing that, serum levels of protease inhibitor could be used in the assessment of prognosis and dissemination of tumor were obtained. We aimed to reveal whether serum alpha-1-antitrypsin (AAT) and alpha-2-macroglobulin (AMG) levels of patients with lung cancer are related with histological type, clinical stage, and advance of cancer. The patients enrolled in the study were the ones with a histopathologic diagnosis of primary lung cancer and they were not administered any treatment before. Levels of serum AAT and AMG were analyzed at the moment of diagnosis. A total of 47 patients were enrolled in the study – 42 males and 5 females. The average age was 58.9. Tumor cell type was non-small cell lung carcinoma (NSCLC) in 36 and small cell lung carcinoma in 11 patients. There was a negative correlation between serum AAT level and age (p= 0.21 r=-0.339). No correlation was identified between serum AMG level and age (p= 0.138 r= 0.222). There was no significant relation between histological types of lung cancer and serums AAT and AMG (p= 0.807, p= 0.411 respectively). AAT and AMG levels were not different in limited and extensive small cell lung carcinoma (p= 0.201 and p= 0.465). AAT and AMG levels based on stages were not found to be different in NSCLC (p= 0.646 and p=0.650). AAT and AMG levels did not vary based on distant metastasis or local spread in NSCLC (p= 0.371 and p= 0.676). Serum alpha-1-antitrypsin and alpha-2-macroglobulin levels in patients with lung cancer do not vary based on histological types, stage of the disease, local, distant and lymphatic spread of the tumor.

Keywords: Lung cancer, Alpha-1-antitrypsin, Alpha-2-macroglobulin, Histology, Stage, Metastasis

ÖZET

Akciğer Kanserinde Serum Alfa-1-Antitripsin ve Alfa-2-Makroglobulin Düzeyleri ile Tümor Histolojisi veya Yaygınlığı İlişkili mi?

Bazı kanser tiplerinde artmış serum proteaz inhibitor düzeyleri saptanmış ve serum proteaz inhibitör düzeylerinin tümörün yaygınlığında, prognozun değerlendirilmesinde kullanılabileceğine dair ipuçları elde edilmiştir. Akciğer kanserli hastalarda serum alfa-1-antitripsin (AAT) ve alfa-2-makroglobulin (AMG) düzeyleri ile kanserin histolojik tipi, klinik evresi ve yaygınlığı arasında ilişki olup olmadığını ortaya koymayı amaçladık. Histopatolojik olarak primer akciğer kanseri tanısı konulan, herhangi bir tedavi uygulanmamış hastalar çalışmaya alındı. Teşhis anında serum AAT ve AMG düzeyleri incelendi. Çalışmaya 42 erkek ve 5 kadın, toplam 47 hasta alındı. Yaş ortalaması 58,9 yıl idi. Tümör hücre tipi 36 hastada küçük hücreli dışı akciğer karsinomu (KHDAK) ve 11 hastada küçük hücreli akciğer karsinomu idi. Serum AAT düzeyi ve yaş arasında negatif korelasyon var idi (p= 0.21 r=-0,339). Serum AMG düzeyi ve yaş arasında korelasyon saptanmadı (p= 0.138 r=0.222). Akciğer kanserinin histolojik tipleri ile serum AAT ve AMG arasında anlamlı ilişki saptanmadı (sırasıyla p= 0.807, p= 0,411). Küçük hücreli akciğer karsinomunda sınırlı ve yaygın hastalıkta serum AAT ve AMG düzeyleri farklı değil idi (p= 0.201 ve p= 0.465). KHDAK'de evrelere göre AAT ve AMG düzeyleri farklı bulunmadı (p= 0.646 ve p= 0,650). KHDAK'de uzak metastaz veya lokal yaygınlığa göre AAT ve AMG düzeyleri farklılık göstermedi (p= 0.371 ve p= 0.676). Akciğer kanserli hastalarda serum alfa-1-antitripsin ve alfa-2-makroglobulin düzeyleri histolojik tiplere, hastalığın evresine, tümörün lokal, uzak ve lenfatik yaygınlığına göre farklılık göstermemektedir.

Anahtar Kelimeler: Akciğer kanseri, Alfa-1-antitripsin, Alfa-2-makroglobulin, Histoloji, Evre, Metastaz

INTRODUCTION

Although partially characterized low molecular weight inhibitors exist in respiratory tract secretions, the most important components of antielastase regulator system are alpha-1-antitrypsin (AAT), and antileucoprotease (ALP) in the lung and AAT and alpha-2-Macroglobulin (AMG) in serum.[1]

Alpha-1-antitrypsin

Trypsin is an antiprotease that can bind collagenase, elastase and plasmin. In certain types of cancer, increased serum levels of AAT were found. The balance between proteolysis and inhibition of proteolysis is disrupted in pathological cases such as tumor formation.[2] Tumor progression is often associated with extensive tissue remodeling. This prepares a more suitable environment for the growth, invasion and metastasis of tumor. Proteinases released from cancer and / or normal tissue cells are known to play a key role in this case.[3] Findings from several studies show that protease inhibitor levels are correlated with tumor stage in patients with primary lung cancer and response to the treatment.[4] Alpha-1-antitrypsin can penetrate many tissues due to its suitable molecular weight. However, its concentration in these tissues is low. For example, AAT level on the epithelial side of respiratory system is 3-4 μm, 10% of the level of serum.[5] AAT – which is an acute phase protein – takes part in tissue transformation, (turnover) coagulation, complement activation and inflammation-induced proteolytic events. Its main physiological function is inhibition of neutrophil elastase.[6,7] AAT is mainly produced in the liver; however it is also synthesized by blood monocytes and bronchoalveolar macrophages. Smoking causes accumulation of polymorphonuclear leukocytes and macrophages in the lungs. Oxidant agents released by stimulation of these cells and caused by tobacco smoke (superoxide radicals, peroxide, polymeric phenoxy radicals, oxiradicals, etc.) reduce the inhibitory capacity of methionine by causing oxidation of the antiprotease located in the AAT reactive centre. Thus, anti-elastase capacity of AAT's neutrophil elastase decreases 2000 times.[8,9]

Alpha-2-macroglobulin

It is another serum inhibitor of neutrophil elastase. It is responsible for 10% of the capacity of serum antielastase[10] AMG cannot penetrate tissues due to its high molecular weight but it is inhibitor effective on proteases directly released to plasma. Though a certain amount of AMG is produced by macrophages in lungs, contribution of that amount is very little to antielastase system in lung tissue.[1]

AMG-protease complex formed during circulation are cleared from circulation by being taken into cells through receptors that can be seen on surfaces of several cells (hepatocytes, macrophages, fibroblasts, and neurons in the central nervous system).[11,12]

In this study we aimed to reveal whether a relation exists between serum alpha-1-antitrypsin, alpha-2-macroglobulin levels and histological type, local – metastatic spread of cancer or not.

MATERIALS AND METHODS

Patients enrolled were histopathologically diagnosed with primary lung cancer and not administered any treatment before. Patients with chronic illnesses that may affect AAT and AMG levels such as tuberculosis, chronic obstructive pulmonary disease, liver failure, the presence of extrapulmonary malignancy were excluded.

All the patients were evaluated by physical examination, standard chest radiography, computed tomography of the chest and upper abdomen. They were diagnosed with lung cancer with fiberoptic bronchoscopy and / or transthoracic fine needle aspiration biopsy. Bone scintigraphy for staging, computed brain tomography and all abdominal ultrasonography were used. While clinical staging, the binary system which Veterans Administration Lung Cancer Group (VALG) recommended for small cell cancer was used; and as for other cancer types, TNM classification of American Joint Committee on Cancer (AJCC) was used.

Venous blood samples were taken from patients after 12 hours of hunger and at least 20 minutes of rest in the sitting position. Serum samples separated after centrifugation were kept under a temperature of -20^{0}C in our hospital microbiology laboratory until the test was conducted. AAT and AMG levels were studied with Beckman Coulter Image Immunochemistry System device by nephelometric method.

The study was approved by the Hospital Scientific Committee. Written consent was taken from all patients participating in the study.

Statistics

All data analysis was performed using SPSS 13.0 software package. The distribution of data was assessed by Kolmogorov-Smirnov test. One-sided analysis of variance was applied to the data fit a normal

Table 1. Selected characteristics of patients with lung cancer

	n	%
Average Age (Years)	58.9±10.5	
Gender		
Male	42	89.4
Female	5	10.6
Age Range (Years)		
33-45	4	8.5
46-55	14	29.8
56-65	15	31.9
66-75	14	29.8
Histological Type		
NSCLC (subtype unknown)	13	27.7
Squamous cell carcinoma	12	25.5
Adenocarcinoma	10	21.3
Large cell carcinoma	1	2.1
SCLC	11	23.4
Smoking (pack year)		
Non - Smoker	3	6.4
1-10	3	6.4
11-20	4	8.5
21-30	7	15
31-40	8	17
41-50	9	19.1
51-60	4	8.5
61 and above	9	19.1
NSCLC Stage		
IA	1	2.1
IB	3	6.4
IIA	1	2.1
IIIA	3	6.4
IIIB	12	25.6
IV	16	34.0
SCLC Stage		
Limited	5	10.6
Extensive	6	12.8
Distant Organ Metastases	21	46.8
Bone	8	17.0
Liver	5	10.6
Brain	7	14.9
Adrenal	4	8.5
Skin	1	2.1

NSCLC: non-small cell lung carcinoma:
SCLC: small cell lung carcinoma

distribution. Bonferroni analysis was applied to variants with homogeneous variance in post hoc evaluation; and Tamhane analysis was applied to those with non – homogeneous data. In the analysis of the data did not fit a normal distribution; the Mann-Whitney U test was used, $p< 0.05$ was considered significant.

RESULTS

General characteristics of 47 patients included in this study are listed in Table 1. While negative correlation was found between age and the level of AAT, there was no correlation with the level of the AMG. No correlation was identified between smoking (pack year) and AAT levels; however, a positive correlation was identified between AMG levels (Table 2). Levels of AAT and AMG did not vary according to histological types (Table 3). Levels of AAT and AMG did not vary in patients with NSCLC (Table 4). No difference was spotted in the AAT and AMG levels according to local, distant and lymphatic spread of tumor in patients with NSCLC (Table 5).

DISCUSSION

Serum alpha-1-antitrypsin and alpha-2-macroglobulin levels in patients with lung cancer do not vary based on histologic types, stage of the disease, local, distant and lymphatic spread of tumor.

Protease and antiproteases are normally two balanced opposing compartments. This imbalance between these two compartments results in tissue damage. And this creates a favorable environment for carcinogenesis and tumor progression. Laboratory research and clinical findings show that AAT deficiency is related with increased liver cancer, gallbladder cancer, bladder cancer, lymphoma, and lung cancer. But nonetheless, increased neutrophil elastase levels facilitate the formation, invasion, and metastasis of many cancers.[13] Lack of protease inhibitors in malignancies may be due to increased protease activity of malignant cells. Granulocyte infiltration around tumor consumes protease activity. In a study conducted by Dobrowska et al., levels of AAT, AMG and alpha-1-antikimotripsin were evaluated and serum AMG and AAT levels were found to be higher than that of control group. There was no significant change in the levels of alpha-1-antikimotripsin.[14]

Disorders of blood coagulation were detected in patients with cancer, and it is shown that this might be due to serum proteinase inhibitors including AAT and AMG.[15] In this study which compared patients with

Table 2. The Correlation of AAT and AMG levels with patients' characteristics.

	Age (year)	Smoking (pack year)			Mean
	r	p	r	p	
AAT (mg/dl)	-0.339	0.021	-0.109	0.262	161.5±131.3 (32-480)
AMG (mg/dl)	0.222	0.138	0.023	0.814	259.6±68.8 (140-430)

AAT: serum alpha-1-antitrypsin levels; AMG: serum alpha-2-macroglobulin levels

Table 3. AAT and AMG levels by histological types

	Histology	n	Mean (mg/dl)	p
AAT	NSCLC (Unknown subtype)	13	146.3±106.9	
	SCLC	11	147.7±142.2	0.807
	Squamous	12	193.7±155.6	
	Adenocarcinoma	10	157.8±10.4	
	Total	46	161.5±131.3	
AMG	NSCLC	13	234.2±52.1	
	SCLC	11	276.5±72.4	0.411
	Squamous	12	273.7±77.1	
	Adenocarcinoma	10	257.3±73.6	
	Total	46	259.65	

NSCLC: non-small cell lung carcinoma; SCLC: small cell lung carcinoma; AAT: serum alpha-1-antitrypsin levels; AMG: serum alpha-2-macroglobulin levels

Table 4. AAT and AMG levels in SCLC

	General	Limited	Extensive	p
AAT (mg/dl)	147.7±142.2	129.3±159.3	169.8±132.9	0.201
AMG (mg/dl)	276.6±72.4	296.2±84.3	253.0±54.3	0.465

AAT: serum alpha-1-antitrypsin levels; AMG: serum alpha-2-macroglobulin levels

inoperable lung cancer with the ones with non-malignant pulmonary disease; lung cancer group had significantly higher serum levels of AAT, and there was no difference in the levels of AMG. When histologic types were compared with each other, it was seen that patients with NSCLC had significantly high serum AAT levels but no significant difference was found in AMG level.[16] When AAT expression in tumor cells in patients diagnosed with adenocarcinoma of the lung and operated was analyzed, it was determined that patients with pathologic stage II and IIIA and IIIB had significantly higher expression than patients with pathologic stage I. No correlation between T factor and AAT immunoreactivity was determined. More positivity was found in patients with nodal involvement than those without involvement. It was noted in the study that strong AAT immunoreactivity may indicate high malignancy of lung adenocarcinomas and poor prognosis.[17] In our study, levels of serum AAT and AMG did not vary significantly based on histologic types of tumor.

Kasprzyk et al., analyzed the levels of acute-phase proteins in 46 NSCLC patients scheduled for curative resection. It was found that serum levels of AAT were significantly higher in patients with adenocarcinoma than other histologic types. There was no significant difference in acute phase proteins by stage of lung cancer. When local tumor dissemination was

Table 5. AAT and AMG levels by the presence of local, lymphatic dissemination and distant Metastasis in NSCLC

Factor	n	AAT (mg/dl)	AMG (mg/dl)
T (Tumor) Factor			
T4	23	157.8±115.1	251.1±73.6
T1-2-3	13	184.4±152.0	260.6±55.4
	p	1.0	0.365
N (Lymph Node) Factor			
N0	10	184.8±136.9	228.9±33.7
N1-2-3	26	160.7±126.7	264.4±74.1
	p	0.751	0.230
Distant Metastasis			
Yes	17	187.9±137.6	259.6±84.5
None	19	149.1±119.7	250.1±48.2
	p	0.371	0.676
Stage			
I-II-IIIA	8	172.8±169.6	266.5±55.8
IIIB	12	139.4±71.7	240.1±39
IV	16	185.8±141.8	259.4±87.2
	p	0.646	0.650

taken into account, it was determined that levels of c-reactive protein, seruloplasmin, alpha-1-antitrypsin, alpha-2-macroglobulin, alpha-1-antikimotripsin, haptoglobin and transferrin were significantly increased. The conductors stated that AAT might be a sign of cancer and can be used as a marker of cancer relapse.[18] When Zelvyte et al. compared AAT levels of patients with lung cancer 14 of which were local spread and 18 of which had distant metastases, they found that patients with distant metastases had significantly higher levels of serum AAT. They marked that tumor cells released serine protease themselves and this extra release facilitates tumor progression.[19] Unlike this, we did not find any relations between levels of AAT and AMG and the stage and local (T-factor), distant (M factor) or lymphatic (N factor) dissemination of illness in our patients in the serum samples at the time of diagnosis. Serum levels of our patients with distant metastasis were similar to those without metastasis.

A limited number of studies reported that levels of serine protease inhibitor increased in cancer types including breast, gastric, colorectal, and lung cancers. The relation between protease inhibitors, lung cancer and other cancers could not be stated with certainty.[20,21] In our study, a significant relation was not determined between the stage, histology and dissemination of tumor and serum levels of AAT and AMG in patients with lung cancer. A negative correlation between Alpha-1-antitrypsin and age is determined as well as a positive correlation between Alpha-2-macroglobulin and smoking (pack year). A limitation of the study is comparison of little number of patients in histological tumor sub-groups and stage groups. Also, patients with lung cancer were not compared with a control group consisting of benign lung diseases.

In conclusion, serum alpha-1-antitrypsin and alpha-2-macroglobulin levels measured at the time of diagnosis of patients with lung cancer are not related with spread of disease (Factors T, N and M) and tumor histology.

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