Original Article

Determination of alpha 1-antitrypsin levels and of the presence of S and Z alleles in a population of patients with chronic respiratory symptoms*

Avaliação da concentração de alfa 1-antitripsina e da presença dos alelos S e Z em uma população de indivíduos sintomáticos respiratórios crônicos

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Abstract

Objective: To determine the levels of alpha-1 antitrypsin (AAT) and the presence of S and Z alleles in patients with chronic respiratory symptoms. **Methods:** Patients with chronic cough and dyspnea were submitted to clinical evaluation, pulmonary function tests, high-resolution computed tomography, nephelometric determination of AAT and determination of S and Z alleles by polymerase chain reaction. Smoking and AAT levels were considered the dependent variables. **Results:** Of the 89 patients included in the study, 44 were female. The mean age was 51.3 ± 18.2 years. The S and Z alleles were detected in 33.3% and 5.7%, respectively, and the gene frequency was 0.16 and 0.028, respectively. Two patients were SZ heterozygotes (AAT levels ≤ 89 mg/dL). The patients were divided into groups based on AAT level: ≤ 89 mg/dL (deficiency, no group); 90-140 mg/dL (intermediate, Group 1, n = 30); and ≥ 141 mg/dL (normal, Group 2, n = 57). The frequency of smokers was the same in both groups, although tobacco intake was greater in Group 2. The S allele was present in 13 and 14 patients in Groups 1 and 2, respectively, whereas the Z allele was present in 2 and 1 patient in the same groups. There was no difference in the results of pulmonary function tests or in the frequency of bronchiectasis or emphysema between the two groups. Spirometric values and AAT levels were similar in smokers and nonsmokers. Bronchiectasis was more common in nonsmokers, and emphysema was more common in smokers. **Conclusions:** Thirty patients presented AAT levels lower than the mean values found in patients with the MM or MS genotype, and this fact could not be explained by an increased frequency of S and Z alleles.

Keywords: Alpha 1-antitrypsin; Emphysema: Lung diseases; Alleles.

Resumo

Objetivo: Determinar a concentração de alfa 1-antitripsina (AAT) e a prevalência dos alelos S e Z em indivíduos sintomáticos respiratórios crônicos. **Métodos:** Pacientes com tosse crônica e dispnéia foram submetidos à avaliação clínica, espirometria, tomografia computadorizada de tórax, dosagem de AAT por nefelometria e pesquisa das mutações S e Z por reação em cadeia da polimerase. Foram consideradas como variáveis dependentes a concentração de AAT e o tabagismo. **Resultados:** Dos 89 pacientes incluídos no estudo (44 mulheres; idade média, 51,3 ± 18,2 anos), os alelos S e Z foram detectados em 33,3% e 5,7%, respectivamente, com freqüência gênica dos alelos S e Z de 0,16 e 0,028. Dois pacientes tinham genótipo SZ (AAT ≤ 89 mg/dL). Os pacientes foram divididos em grupos segundo a concentração de AAT: ≤ 89 mg/dL (deficiência, nenhum grupo); 90-140 mg/dL (faixa intermediária, Grupo 1, n = 30); e ≥ 141 mg/dL (normal, Grupo 2, n = 57). A freqüência de fumantes foi igual nos dois grupos, com carga tabágica maior no Grupo 2. O alelo S estava presente em 13 e 14 pacientes dos Grupos 1 e 2, respectivamente, enquanto que o alelo Z estava presente em 2 e 1 paciente dos mesmos grupos. Não houve diferença nos testes de função pulmonar, nem na freqüência de bronquiectasias ou enfisema entre os dois grupos. Os valores espirométricos e as concentrações de AAT foram similares entre fumantes e não-fumantes. Bronquiectasias foram mais freqüentes entre os não fumantes, e enfisema foi mais freqüente entre os fumantes. **Conclusões:** Trinta pacientes apresentaram níveis de AAT abaixo da média esperada para os genótipos MM e MS, e este fato não pode ser explicado por uma freqüência maior dos alelos S e Z.

Descritores: Alfa 1-antitripsina; Enfisema; Pneumopatias; Alelos.

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Introduction

Neutrophils, which normally pass unimpeded through the pulmonary vascular system, adhere to the endothelium of capillaries and venules in response to activation by inflammatory stimuli. Neutrophil activation leads to the release of many substances, such as reactive oxygen species, cationic peptides, eicosanoids and proteolytic enzymes, all of which have the function of destroying aggressor agents. The activity of those substances, however, needs to be controlled by antagonists in order to prevent tissue damage and organ dysfunction.

Serine proteases, a group of neutrophil proteolytic enzymes, have various functions, such as degrading elastic and collagen fibers, producing secretory cell metaplasia in the respiratory epithelium, affecting the ciliary beat, activating the complement system, increasing interleukin-8 synthesis and increasing tumor necrosis factor-alpha synthesis, as well as activating or inactivating various cytokines.[1]

Neutrophil elastase, which is the most important serine protease, is one of the few human enzymes capable of degrading elastic fibers, which are responsible for tissue elastic recoil, including that of the alveolar walls.[2]

Clinical and experimental lines of evidence have suggested that emphysema is caused by elastase-induced destruction of elastic fibers of the lung interstitium. In 1963, two authors described five patients with alpha 1-antitrypsin (AAT) deficiency, AAT being the substance primarily responsible for neutrophil elastase inhibition; three of those patients had emphysema.[3] In 1965, one group of authors instilled papain (through the trachea) into the lung of rodents in an attempt to produce granulomas.[4] The authors discovered that papain caused emphysema.

Circulating at levels ranging from 120 to 200 mg/dL (measurements obtained by nephelometry), AAT is the principal inhibitor of serum protease. Significant reductions in serum and tissue AAT levels in human beings are associated with chronic obstructive pulmonary disease (COPD), in particular, as well as with pulmonary emphysema, with liver disease, which can lead to cirrhosis, necrotizing panniculitis and vasculites presenting positivity for antineutrophil cytoplasmic antibodies with cytoplasmic pattern.[5]

The gene responsible for AAT synthesis is located on chromosome 14, and production occurs principally in hepatocytes, although other cells, such as mononuclear phagocytes and epithelial cells of the lung and intestine, can contribute. The highly pleomorphic nature of AAT indicates that the gene locus is also highly variable: approximately 100 different alleles have been identified to date. The variants are inherited codominantly and are classified according to the protease inhibitor (PI) system, which is based on the electrophoretic mobility of the different proteins on acrylamide gels. The most common phenotype, the MM (medium mobility) phenotype, is present in 94% to 96% of Caucasians and is associated with serum AAT levels within the 150-350 mg/dL range.[5]

Low serum and tissue levels of AAT occur as a result of the inheritance of two alleles (S and Z) that encode decreased levels of this protein.

The Z allele (PI*Z), when homozygous (PI*ZZ), results in serum AAT levels ranging from 15 to 50 mg/dL. In this setting, panacinar pulmonary emphysema is the most common clinical manifestation, as well as being the leading cause of incapacity and death. The Z allele is the allele most commonly found in Caucasians coming from northern Europe, and accounts for 1-3% of all AAT alleles in such individuals.[2]

The S allele is more common than the Z allele, accounting for 2-4% of all AAT alleles in Caucasians from northern Europe and for 15% of all AAT alleles in individuals from the Iberian Peninsula, being especially prominent in those from northern Portugal and the region of Galicia, in Spain.[2,6]

The SZ genotype (PI*SZ) results in AAT levels ranging from 45 to 105 mg/dL, whereas the SS genotype (PI*SS) produces levels ranging from 100 to 140 mg/dL.[7] Individuals who are SZ heterozygotes are three times more likely to develop COPD.[8]

The low frequency of S and Z alleles in the population makes it difficult to obtain data on the gene frequency and the presence of AAT deficiency in the general population. In Brazil, given the level of laboratory sophistication required, there have been no studies involving epidemiological investigation in the general population.

These facts have raised interest in determining the presence of S and Z alleles in the population of patients with chronic respiratory symptoms, without asthma, treated at the outpatient clinic of the Department of Pulmonology of a regional referral hospital.

The objective of the present study was to determine serum AAT levels, as well as to identify S and Z alleles, in a population of patients with chronic respiratory symptoms.

Methods

We selected patients with a clinical profile of productive cough and dyspnea treated at the outpatient clinic of the Department of Pulmonology. Infectious causes, such as tuberculosis and fungal infections, were ruled out by testing sputum samples, and asthma was ruled out based on clinical history and spirometry results.

Clinical data (age, gender, race, history of smoking and tobacco intake) were collected, and patients were submitted to spirometry, high-resolution computed tomography of the chest, determination of AAT levels and determination of S and Z alleles.

Pulmonary function tests were performed using a spirometer (model AM 4000 PC; Anamed, São Paulo, Brazil). Forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and the ratio between the two (FEV_1/FVC) were analyzed.

High-resolution computed tomography (using thin slices and a high-resolution algorithm) was performed with a GE-9800 scanner (General Electric Medical Systems, Milwaukee, WI, USA) or a SOMATON AR.T scanner (Siemens Medical Solutions, Munich, Germany). The department radiologist evaluated the scans for the presence of emphysema or bronchiectasis. No quantitative analysis of the lesions was performed, nor was the extent or severity of the lesions estimated.

The presence of S and Z alleles was determined by polymerase chain reaction, followed by enzymatic digestion of this amplified material with the restriction enzymes XmnI (S allele) and TaqI (Z allele).[9] After the amplification of the DNA fragment and its enzymatic digestion, the samples were submitted to electrophoresis on a 16% polyacrylamide gel.

Nephelometric determination of serum AAT levels was performed using the Array 360 System (Beckman Instruments, Inc., Fullerton, CA, USA). The normal reference range for this test is from 90 to 200 mg/dL.

The data obtained for each patient were analyzed in two ways: by using AAT levels as a dependent variable and by stratification based on the presence/absence of smoking.

The project was approved by the Ethics in Research Committee of the institution, and all patients gave written informed consent prior to their enrollment in the study.

Table 1 - Clinical characteristics and spirometric parameters, as well as results of the determination of alpha-1 antitrypsin levels and of the presence of S and Z alleles, in the patients evaluated.

Characteristic[a]	Group 1	Group 2	p
Patients, n	30	57	
Age (years)	49.0 ± 17.9	52.1 ± 18.7	0.383
Smokers, n	17	33	0.9123
Tobacco intake (pack-years)	28.1 ± 20.4	45.4 ± 27.4	0.026
AAT levels (mg/dL)	121.6 ± 12.90	176.8 ± 35.80	0.0001
FVC, % of predicted (L)	60.5 ± 18.6	64.9 ± 21.5	0.340
FEV_1, % of predicted (L)	41.8 ± 18.0	47.3 ± 21.9	0.393
VEF_1/CVF	54.6 ± 14.1	58.0 ± 16.5	0.557
S allele, n/total	13/28	14/57	0.072
Z allele, n/total	2/28	1/57	0.272

[a]Values expressed as mean ± standard deviation, except when specified; Group 1: alpha-1 antitrypsin (AAT) levels of 90-140 mg/dL (intermediate); Group 2: AAT levels ≥ 141 mg/dL (normal), FVC: forced vital capacity; and FEV_1: forced expiratory volume in one second.

Statistical analysis of continuous data was performed using the parametric Student's t-test or the nonparametric Wilcoxon test, when necessary. The correlations among categorical variables were studied using a parametric chi-square test or the nonparametric Fisher's exact test, depending on the distribution of frequencies in a two-by-two table. The correlations among continuous variables were analyzed using Pearson and Spearman correlation tests. The SAS® (Statistical Analysis System, Cary, NC, USA) and the Minitab® (Minitab Inc., State College, PA, USA) statistical software were used for all tests.

The level of statistical significance was set at 5%.

Results

A total of 89 patients (44 females and 45 males) were evaluated. The mean age was 51.3 ± 18.2 years. Of those, 87 were submitted to determination of S and Z alleles. The S allele was detected in 29 (33.3%) of the 87 patients evaluated, whereas the Z allele was detected in 5 (5.7%). The gene frequency of S and Z alleles was 0.160 and 0.028, respectively. The sample is in Hardy-Weinberg equilibrium ($\chi^2 = 2.69$; $p = 0.61$), which indicates that it is not subject to selection pressure and that the migration flow and the mutation rate are not significant to the point of affecting the interpretations of the analyses performed.

For the analysis of the results, the patients were divided into groups based on AAT levels, according to the cut-off values suggested in the guidelines for the diagnosis and management of individuals with AAT deficiency established by the American Thoracic Society (ATS) in collaboration with the European Respiratory Society (ERS)[5]: ≤ 89 mg/dL (deficiency); 90-140 mg/dL (intermediate); and ≥ 141 mg/dL (normal).

Only 2 patients presented AAT levels ≤ 89 mg/dL, a number that is insufficient for statistical analysis. Both of those patients were SZ heterozygotes, were heavy smokers (18 and 20 pack-years) and had COPD. One of the two patients had chronic respiratory insufficiency and used home oxygen therapy, whereas the other had a history of frequent exacerbations and presented bronchiectasis on the tomography scan. Among the 89 patients, AAT levels were 90-140 mg/dL in 30 and ≥ 141 mg/dL in 57. These were designated Group 1 and Group 2, respectively.

The clinical characteristics, AAT levels, spirometry findings and tomographic findings, as well as the results of the determination of S and Z alleles, for Groups 1 and 2 are listed in Table 1.

There was no difference in terms of the percentage of smokers (57% and 58% in Groups 1 and 2, respectively), although tobacco intake was significantly different ($p = 0.026$), being greater in Group 2 (45 pack-years) than in Group 1 (28 pack-years).

The S allele was present in 13 and 14 patients in Groups 1 and 2, respectively, compared with 2 patients and 1 patient, respectively, for the Z allele. There was no significant difference between the two groups in terms of the presence of S or Z alleles ($p = 0.072$ and $p = 0.272$, respectively).

The comparison between Groups 1 and 2 in terms of tomographic findings revealed no differences in the frequency of bronchiectasis ($p = 0.324$) or emphysema ($p = 0.938$).

The analysis of AAT levels of the patients in Groups 1 and 2, taken together, in the presence or absence of the S allele (Table 2), revealed a significant difference in the medians ($p = 0.0089$), with greater values in the group without the S allele.

A second analysis, considering all of the patients studied, compared smokers and nonsmokers in terms of AAT levels and spirometry results. No differences were found in any of the parameters (AAT levels, $p = 0.585$; FVC, $p = 0.157$; FEV_1, $p = 0.870$; and FEV_1/FVC, $p = 0.134$). However, smoking was found to be associated with presence of bronchiectasis and emphysema, in opposite ways: bronchiectasis was more common in nonsmokers ($p = 0.008$) and emphysema was more common in smokers ($p = 0.032$).

Discussion

Since this was a cross-sectional cohort study involving a population of patients with respiratory symptoms, the data obtained cannot be extrapolated to the general population. The present study

Table 2 - Alpha-1 antitrypsin levels in the presence or absence of the S allele.

Variable	S allele	Level, median (minimum-maximum)	p
AAT (mg/dL)	Present	143 (105-181)	0.0089
	Absent	166 (97-371)	

AAT: alpha-1 antitrypsin.

was carried out in an institution that serves a region with approximately 4 million inhabitants, and, since this institution is a referral hospital for severe cases, the population treated represents the characteristic miscegenation of the Brazilian population, especially that of the state of São Paulo.

In the analysis of the results, we opted for using three serum AAT level ranges to define three groups: the first with AAT levels ≤ 89 mg/dL (deficiency); the second with AAT levels of 90-140 mg/dL (intermediate); and the third with AAT levels ≥ 141 mg/dL (normal). According to the ATS/ERS document,[5] even patients with intermediate AAT levels should be submitted to a qualitative test, such as phenotyping or genotyping, as was done in the present study, although AAT levels > 50 mg/dL are considered protective against the development of emphysema.

Decreased serum AAT levels should not have, a priori, any relationship with the smoking habit. In fact, since AAT is an acute phase protein, the expected effect of smoking on AAT levels would be that of an increase, due to the inflammatory process triggered by the substances present in tobacco smoke. There is evidence that, although not decreasing AAT levels in the blood and tissues, smoking can decrease AAT activity due to methionine oxidation at the protein active site.[5] In the patients studied here, greater tobacco intake was associated with higher AAT levels (p = 0.026).

In our sample of 89 patients with chronic respiratory symptoms, 30 (33.7%) were classified as having intermediate AAT levels, with mean serum levels of 121.6 ± 12.9 mg/dL, below the means predicted for individuals with the MM or MS alleles (149.1 ± 38.2 mg/dL and 150.0 ± 40.0 mg/dL, respectively).[10] In this group, designated Group 1, tobacco intake was significantly lower than that found in the group of patients with normal AAT levels. Considering the patient inclusion criteria adopted in the present study (chronic complaints of productive cough and dyspnea), we can infer, based on this finding, that patients with intermediate AAT levels had chronic respiratory symptoms, although they smoked less heavily than did the 57 individuals (64%) with normal AAT levels (Group 2, mean AAT levels of 176.8 ± 35.8 mg/dL).

In addition, the two groups presented equal degrees of abnormality in the spirometry findings (p > 0.05). In Group 1, FVC (% of predicted) was 60.5 ± 18.6 L, FEV_1 (% of predicted) was 41.8 ± 18.0 L and the FEV_1/FVC ratio was 54.6 ± 14.1, compared with 64.9 ± 21.5 L, 47.3 ± 21.9 L and 58.0 ± 16.5, respectively, in Group 2 (Table 1).

Based on the data presented above, we can conclude that, in the population studied, intermediate AAT levels were more common among patients who smoked less heavily. However, the intermediate AAT levels cannot be explained by a greater presence of S and Z alleles, since there was no statistically significant difference between the two groups in terms of the frequency of S and Z heterozygotes, which was 13/28 and 14/57 for S heterozygotes in Groups 1 and 2, respectively, compared with 2/28 and 1/57, respectively, for Z heterozygotes (Table 1; p = 0.072 and p = 0.272, respectively). Nevertheless, it should be noted that the frequency of the S allele was greater in Group 1 than in Group 2, and the level of significance found in the comparison of the two groups (p = 0.072) seems to suggest a tendency that would eventually be confirmed if we had evaluated a greater number of patients.

In a meta-analysis published in 2005,[8] the role of the S allele in the risk of COPD was investigated. The authors found that SZ heterozygotes were three times more likely to develop the disease. Regarding individuals with the MS genotype, cross-sectional and case-control studies have revealed a small but significant increase in the risk of COPD.

In addition, the median AAT levels were significantly lower in patients with a single S allele (p = 0.0089) than in patients without the S or the Z allele (Table 2). In the present study, lower AAT levels, whether caused by the presence of a deficient allele or not, seemed to be associated with higher susceptibility to respiratory diseases. Since only S and Z alleles were investigated, we cannot rule out the possibility that patients with intermediate AAT levels have some known rare allele or even genetic alterations yet to be described, which could be detected only by gene sequencing.

There are nongenetic causes of AAT deficiency, such as liver diseases or other pathological conditions that lead to protein loss. However, those comorbidi-

ties were not present in the patients included in the present study.

The frequency of bronchiectasis was the same in Groups 1 and 2, as it was in patients with and without the S allele. Although there is substantial evidence that the presence of bronchiectasis is common in patients with severe AAT deficiency,[11] the genesis of bronchiectasis is certainly multifactorial.

Considering smokers and nonsmokers, tomographic findings of bronchiectasis were significantly more common in nonsmokers, probably because this disease was the cause of chronic respiratory symptoms that motivated the inclusion of those patients in the study.

As expected, tomographic findings of emphysema were much more common in smokers than in nonsmokers, although there was no statistically significant difference between Groups 1 and 2 in terms of the frequency of a diagnosis of emphysema.

Low AAT levels can worsen emphysema lesions due to the lack of protease inhibition, as well as to the absence of inhibition of alveolar epithelial cell apoptosis. In an experimental study involving a model of noninflammatory emphysema,[12] in which the alterations were produced by the inhibition of vascular endothelial growth factor receptors, the authors provided additional evidence of this new role of AAT (reducing alveolar epithelial cell apoptosis in emphysema). In mice more susceptible to tobacco-induced emphysema, which present a 50% reduction in AAT levels in the bronchoalveolar lavage fluid, the increase in the AAT levels available decreased the emphysema produced by the vascular endothelial growth factor receptor inhibitors.

However, in the airways, uninhibited proteases can injure the bronchial epithelium, producing secretory metaplasia and hypersecretion.[1] Even a slight decrease in AAT levels, such as that observed in individuals with the MZ genotype, can increase the risk of developing pulmonary diseases,[13] and the impaired function of AAT denatured by oxidation in smokers (even in those presenting normal AAT levels) can contribute to the development of lung injury.[5] These facts seem to indicate that AAT availability is extremely important for the protection of the lung and, probably, for the maintenance of the adequate state of many other organs and systems.

In summary, the S allele was detected in 33.3% of the 89 patients studied, whereas the Z allele was detected in 5.7%. The quantitative analysis of AAT levels revealed that 30 patients presented serum AAT levels lower than the mean values found in patients with the MM or MS genotype, who account for most of the population (94-96% of Caucasians).[5]

In Brazil, there are no data on the frequency of S and Z alleles or on the quantitative analysis of AAT levels in the general population. It can be said, however, that the AAT levels found, which were classified as intermediate, cannot be explained by an increased frequency of S and Z alleles in those patients. More comprehensive studies, preferably multicenter studies, are needed in order to determine the frequency of the principal alleles associated with AAT deficiency in Brazil.

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