A New Variant of Alpha-1-Antitrypsin Deficiency (Siiyama) Associated with Pulmonary Emphysema

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A 38-year-old male with pulmonary emphysema due to severely reduced serum alpha-1-antitrypsin (AAT) level (14.5 mg/dl) was found to have an inherited new AAT deficient variant Siiyama. Chest roentgenogram and CT scanning revealed advanced emphysema, and severe obstructive ventilatory impairment was observed. During the 4-year follow-up period, the annual rate of decline of FEV 1.0 showed approximately 10-fold greater than the normal decline in FEV 1.0 (−380 ml/yr). Treatment with tamoxifen in order to raise the serum AAT level only resulted in an insufficient increase. Augmentation therapy of human AAT should be considered in the future.

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Key words: tamoxifen, augmentation therapy, human alpha-1-antitrypsin (AAT)

Introduction

Alpha-1-antitrypsin (AAT) deficiency is a hereditary disorder, accompanied by a high risk for the development of panacinar emphysema and liver disease (1–3). In the United States, about 2% of all pulmonary emphysema has been attributed to AAT deficiency (4). In Japan AAT deficiency is very rare and only a few cases have been reported (5–9).

We report a case with a new type of AAT deficiency accompanied by severe emphysema especially from the viewpoint of clinical consideration.

Case Report

A 38-year-old male pigbreeder was well until at age 30 when he gradually developed dyspnea on exertion. In September 1986, at the occasion of cholelithotomy, obstructive ventilatory impairment (FEV 1.0 2.10 L, FEV 1.0% 38%) was pointed out. He had smoked one pack of cigarettes from age 20 to 34 (smoking index 300). He stopped smoking and has been treated with oral bronchodilators since then. In July 1988 he was referred to Hokushin General Hospital. Because of the early

onset of severe ventilatory impairment and a marked reduction in the alpha-1-globulin peak on serum protein electrophoresis, his serum AAT concentration was examined and found to be below 50 mg/dl.

In April 1989 he was admitted for further evaluation. Physical examination revealed hyperresonance over the chest and diminished breath sounds. Lung function tests demonstrated an increase in lung volumes (VC 5.99 L, 120% predicted, and TLC 8.10 L, 124% predicted) and severe airway obstruction (FEV 1.0% 31.5%), impaired diffusion capacity (DLco/VA 2.94 ml/min/mmHg, 49% predicted) was also noted (Table 1). Arterial blood gases (ABG) showed normocapnic hypoxemia (PaO₂ 65.7 mmHg, PaCO₂ 39.0 mmHg) under room air. Serum protein electrophoresis revealed a reduced alpha-1 globulin peak (0.7%), while other routine laboratory examination data of blood and urine were normal (Table 2). Chest roentgenogram showed hyperinflation, flattened diaphragms, and bullous changes in the lower lung fields in both lungs (Fig. 1A). CT scanning of the chest revealed advanced pulmonary emphysema predominant in the lower lung fields, compatible with panacinar emphysema (Fig. 1B). In bronchoalveolar lavage, neutrophil recruitment in the lower respiratory tract was suspected

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Table 1. Pulmonary Function and Arterial Blood Gas Analysis

Pulmonary Function Test	Arterial Blood Gas				
	1986 ^a	1988 ^b	1989 ^c	Room air	1989 ^c
VC	6.00	5.90	5.97	pH	7.418
%VC	147.0	146.8	150.1	PaCO ₂	39.0 torr
FEV 1.0	2.70	2.07	1.73	PaO ₂	65.7 torr
FEV 1.0%		35.01	31.5	HCO ₃ ⁻	25.1 mEq/l
PEFR			7.04	O ₂ Sat.	93.3%
V50		0.72	0.57		
V25		0.38	0.27		
TLC			8.10		
RV			2.12		
RV/TLC			26.1		
DLco			17.9		
(ml/min/mmHg)					
%DLco/VA(%)			49.0		

^a: data at the time of cholelithotomy (age 35), ^b: data at the time of initial visit to Hokushin General Hospital (age 37), ^c: data at the initial admission to Hokushin General Hospital (age 38).

(Table 2).

Although no apparent liver dysfunction was observed (Table 2), liver biopsy specimens showed PAS-positive, diastase-resistant globules and immunoreactive substances with anti-AAT antibody in several hepatocytes (Fig. 2A, 2B).

Family pedigree revealed consanguinity (Fig. 3). An extremely low serum AAT concentration was observed in the probands (14.5 mg/dl, normal range 148–317 mg/dl). As hereditary disorder was suspected, we examined his family members. Although none showed abnormal lung function tests, all of them had low values in serum AAT (father 106, mother 119, elder sister 87, and daughter 140 mg/dl), indicating the possibility that the patient inherited a deficient AAT gene in a homozygous fashion. The coding exons of the AAT gene were analyzed and a single base substitution from C to T was found in a residue of Ser53 (TCC) changed to Phe53 (TTC) (10). Three generations of the family members

Table 2. Findings of Examinations Including Blood, Urine, Abdominal Ultrasonography and Bronchoalveolar Lavage*

WBC	5,800/ μ l	TP	7.2 g/dl	γ -GTP	14 IU/l
stab	3.0%	Alb	67.1%	ChE	0.85 Δ pH
seg	34.0%	α_1	0.7%		
eos	5.0%	α_2	8.3%	BUN	15.0 mg/dl
baso	3.0%	β	9.5%	Cre	1.0 mg/dl
mono	5.0%	γ -glob	14.4%	Na	145 mEq/l
lym	50.0%	tBil	0.7 mg/dl	K	4.4 mEq/l
RBC	515×10^4 / μ l	GOT	17 IU/l	Cl	108 mEq/l
Hb	16.1 g/dl	GPT	13 IU/l		
Hct	47.8%	ALP	189 IU/l	CRP	<0.3 mg/dl
Plt	26.6×10^4 / μ l	LDH	333 IU/l	α_1 -antitrypsin	<50 mg/dl

Urinalysis: protein (–), sugar (–), sediment; normal

ICG (value of 15 min): 7%

Abdominal ultrasonography and liver scintigraphy: negative finding

Bronchoalveolar lavage: 70% macrophages, 17% neutrophils, 5% lymphocytes

* Abnormal values are underlined.

(his parents, elder sister, and daughter) were found heterozygous in this base, which is consistent with the half-reduced serum levels of AAT in these family members. Taken together, it is probable that this substitution is responsible for the reduced AAT level in this case. With the combination of isoelectric focusing (IEF) and crossed immunoelectrophoresis followed by immunoperoxidase staining, the serum AAT molecule of the proband was found to be migrated to the S position of the reference sera (Fig. 4). We designated this deficient allele as Siiyama after his birthplace.

He was discharged with the treatment of bronchodilators and a face mask that was thought to prevent inhalation of dust during his work of pigbreeding. However, his clinical symptoms and signs were gradually deteriorated; worsened dyspnea on exertion forced him to retire from his work as pigbreeder and FEV 1.0 con-

tinued to decline at the annual rate of –380 ml/yr which is approximately ten-fold greater than the normal decline (Fig. 5). He was readmitted three times because of recurrent left pneumothorax, which improved each time with closed tube drainage.

Due to the rapid deterioration in his clinical course despite the maximal supportive therapy, we started treatment with tamoxifen (20 mg/day) in an effort to raise the serum AAT level. After one month of treatment, the serum AAT level increased from 16 mg/dl to 24 mg/dl, still far below the threshold level (80 mg/dl) (11) and insufficient to protect from the destruction of lung parenchyma.

Discussion

Alpha-1-antitrypsin (AAT) deficiency is an autosomal

Fig. 1. Chest roentgenogram and CT scanning. A) Chest roentgenogram of the proband shows over inflation, flattened diaphragms, and bullous changes, especially in the lower lung fields. B) CT scanning revealed advanced panacinar emphysema with scarcity of pulmonary arteries.

Fig. 2. Light micrographs of the liver ($\times 400$). A) Hematoxylin-eosin staining. Several hepatocytes show PAS-positive and diastase-resistant globules. B) Immunohistochemical staining. Aggregates in the hepatocytes which reacted with anti-AAT antibody are seen.

Fig. 3. A family pedigree of a new AAT deficiency in Japan. The proband is indicated with an arrow. Serum AAT concentration is shown below the symbols. Note the consanguineous marriage of his parents.

Fig. 4. Closed immunoelectrophoretic analysis of AAT Siiyama after IEF. The patterns of M1Z (upper panel), ZZ (middle panel), and the proband (lower panel) are shown. On the top the location of major 4 (m4) and 6 (m6) bands in both cases M1 and Z are indicated. Two broad precipitation bows are observed between M1 and Z, corresponding to the position of S variant.

recessive hereditary disorder characterized by a low concentration of AAT in both the serum and lungs. This disorder is responsible for approximately about 2% of all cases of pulmonary emphysema in the United States. More than 75 alleles of the AAT gene have been identified and classified into the protease inhibitor system (1). The normal and deficient AAT alleles are assigned to a letter code (A to Z) based on the electrophoretic mobility of the molecule on IEF. The most common deficient variant is Z variant, with the incidence of 1 to 2% of the Caucasian population in the United States (1, 12) resulting in about one homozygous individual in 5,000 people. Although the frequency of S allele is more common (2 to 3%), it is less important clinically than Z allele. This is because, in the homozygous form of S allele, the serum AAT level is 100–200 mg/dl, which is sufficient to protect the lungs (1, 11). A serum concentration of 80 mg/dl has been considered a threshold level, below which an individual has an increased risk for the development of pulmonary emphysema (11).

In Japan, due to the lack of the Z allele (13, 14), AAT deficiency is very rare and only 5 cases have been reported (5–9) in the literature. Only one case has been analyzed genetically and was identified as M nichinan, the depletion of Phe52 (TTC) and Gly148 (GGG) — Arg148 (AGG) (15). Recently another case, a 49-year-old female (AAT 70 mg/dl and FEV 1.0% 38%) diagnosed as emphysema with bronchial asthma (7), was identified as the heterozygote of normal M2 and deficient

Fig. 5. Deterioration of pulmonary function in the course of supportive therapy. The decrease rate in FEV 1.0 w as high (–380 ml/yr) even after the cessation of smoking. Dashed line indicates the predicted rate of decline in FEV 1.0 by Berglund et al (26) with the same height as the proband. Note that tamoxifen therapy is not effective to prevent and stabilize the rapid decline in FEV 1.0.

variant M malton, the depletion of Phe52 (TTC) (16). In the present case, a new causative amino acid substitution was identified; its profound effect against the organization of AAT molecule has already been reported by Huber and Carrell (17).

In this disorder, a high risk for the development of liver disease has been also reported. Progressive liver disease with Z variant in childhood is well recognized and aggregation of AAT in hepatocytes has been considered responsible (1). In the present case, immunoreactive-globules with anti-AAT antibody were found in several hepatocytes although no apparent liver dysfunction and other pathological findings on routine histological staining were recognized. Existence of liver inclusion bodies was also observed in M nichinan allele (6).

In addition to the maximal supportive care of pulmonary emphysema, various treatment modalities have been attempted in patients with AAT deficiency. With the knowledge that AAT levels increase during pregnancy, previous studies have been undertaken to raise AAT levels by using tamoxifen, a drug which binds intracytoplasmic estrogen receptors, and danazole, a drug with testosterone-like function. Tamoxifen has been shown to raise deficient AAT levels in patients with S allele (18) and P lowel allele (19), but not with Z allele (20). Danazole had no effect in Z allele patients and had a side effects of hirsutism and fluid retention (21). Due to the rapid deterioration of the present patient's clinical picture and lung function despite the smoking cessation and the effort to eliminate other possible irritants, we considered treatment modalities which might raise the serum AAT level and protect from further lung parenchymal destruction. However, administration of tamoxifen resulted in an insufficient increase of the serum AAT level in Siiyama variant. As a treatment which is theoretically anticipated to be beneficial, AAT augmentation therapy must be considered in the next step.

Since the development of human AAT, the effect of augmentation therapy with human AAT has been extensively studied. The serum AAT level is reported to be maintained above the threshold level by the administration of 60 mg AAT/kg weekly (4, 22). Hubbard et al (23) also reported that aerosolized AAT (200 mg/day) increased AAT concentration in the lung. It has been demonstrated that augmentation therapy is safe and effective in terms of providing serum AAT levels above the threshold protective levels and a functional anti-neutrophil elastase screen on the lower respiratory tract. Based on these advances and still unproved effects in terms of retarding the progression of emphysema, the American Thoracic Society has recently published guidelines for the approach to therapy of patients with AAT deficiency (11). In this article, augmentation therapy was recommended for individuals with phenotypes associated with an AAT serum concentration of less than

80 mg/dl whose pulmonary function is abnormal.

In Japan, AAT deficiency has been recognized as a very rare hereditary disorder. However, an increasing number of patients have been recently reported and the occurrence of 9 deficient families including 14 individuals has been confirmed. Moreover, 5 AAT deficient families including 8 individuals were proved to have Siiyama allele homozygously (manuscript in preparation). As Poller et al (24) noted that clinical course of AAT deficiency is highly variable, the individuals with the same Siiyama allele show different clinical pictures (25), suggesting that additional factors, defects in other genes and environmental influences other than smoking, may be pertinent to the pathogenesis of emphysema associated with AAT deficiency (24). Considering the increasing AAT deficient cases in Japan and the fact that no effective treatment is available AAT augmentation therapy will become necessary in the future. However, the cost of the therapy is more than 2.5 million yen per year and it is not covered by the health insurance in Japan. This is the main reason why these severely ill patients cannot be treated with augmentation therapy at present. Special consideration for the coverage of AAT deficiency augmentation by the Japanese health insurance is desired earnestly.

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