Alpha,-Antitrypsin Pi-Types in 965 COPD Patients

Jack Lieberman, M.D., F.C.C.P.; Benjamin Winter, M.D., F.C.C.P.; and A. Sastre, B.S.

To study further the role of intermediate alpha,-antitrypsin (AAT) deficiency in chronic obstructive pulmonary disease (COPD), AAT Pi-types and serum-trypsin-inhibitory-capacity (STIC) were measured in 965 patients with COPD. Heterozygosity of the Z variant was the major cause of intermediate AAT deficiency (primarily the MZ phenotype), accounting for 8.0 percent of the patients compared to 2.9 percent of control subjects (p<.0005). ZZ homozygosity was detected in 1.9 percent of the patients, compared to 0.04 percent of control studies performed by others (none was present in our own control group of 1,380 subjects). The mean age for MS or MZ patients did not differ from that of the COPD patients as a whole, whereas the ZZ homozygotes were younger (65.9 ± 9.8 vs 65.3 ± 7.5 years). These results resemble those of a previous study in 66 male veterans with pulmonary emphysema suggesting that the MZ phenotype, or intermediate AAT deficiency in general, probably does predispose to the development of COPD. However, the prevalence of AAT deficiency in COPD patients is small (approximately 10 percent). The number with an MS phenotype was not increased in this group of COPD patients.

The association of homozygous (ZZ) alpha,-antitrypsin (AAT) deficiency with pulmonary emphysema and infantile cirrhosis has been firmly established. However, the role of intermediate (heterozygous) AAT deficiency as a predisposing factor for lung disease has been hotly debated since it was first suggested by Lieberman and Kueppers, Fallat and Larson in 1969. Lieberman's initial study involved the measure of serum-trypsin-inhibitory-capacity (STIC) in the blood of 66 veterans, wherein he found 15.2 percent to have intermediate deficient levels. Subsequently, with the advent of Pi-typing, 39 of 47 patients with intermediate-deficient levels of STIC were found to have a heterozygous phenotype containing the Z variant (MZ-33; SZ-5; FZ-I). Thus, in retrospect, approximately 13 percent of the initial 66 veterans with pulmonary emphysema (compared to 2.5 percent of control subjects) had a heterozygous deficiency state involving the Z variant of AAT.

A more recent attempt by Bruce et al to verify the role of the MZ phenotype as a risk for lung disease involved a search for MZ subjects within random populations, questioning them for respiratory symptoms, and testing for pulmonary dysfunction. The authors of this multicenter study concluded that the MZ phenotype alone carries no greater risk of developing lung disease than the normal M phenotype.

An unusual opportunity arose during the years 1976 to 1984 for us to study the STIC and Pi-type of 965 patients with severe COPD referred to one of us (BW) for bilateral carotid body surgery in a desperate attempt to alleviate dyspnea and to improve their quality of life. These patients were a mixed group of COPD patients with emphysema or chronic bronchitis, frequently with an asthmatic component. The study was basically an attempt to confirm our previous findings, but in a much larger patient population utilizing Pi-typing in addition to the measure of STIC. Certain factors could limit the ability of this study to allow a true estimate of the prevalence of antitrypsin deficiency among COPD patients, namely the older age of these patients (AAT deficiency previously was found among younger patients) and the broader diagnostic categories included in the study population (AAT deficiency is usually found more often in patients with pure emphysema). However, if an increase in prevalence of Z heterozygosity or intermediate AAT deficiency were to be found in this population, the significance would be even more meaningful than if the study were limited to a population of emphysema patients alone.

MATERIALS AND METHODS

Patient Selection

Blood samples were obtained from consecutive patients hospitalized for carotid body surgery by BW; serum was separated and delivered to our laboratory at the Sepulveda VA Medical Center. All patients were Caucasian, ranging in age from 41 to 85 years, with a mean ± SD of 65.3 ± 7.5 yrs; 25 percent of the patients were women. All had far-advanced COPD with severe dyspnea as their main complaint. Information such as age of onset of lung disease was not available.

STIC Assay

STIC was assayed essentially as described.44 Crystalline trypsin was kindly provided by the Reheis Chemical Company, Phoenix, Arizona. The absolute protein concentration of the trypsin solution in mg per ml of 0.0025 M HCl was determined by measurement of the absorbance at 280 nm, with multiplication by the conversion factor.
Results

Prevalence of Abnormal Phenotypes

Two separate formats were used for evaluating the Pi-type of these sera. Initially, for the first 223 specimens, phenotyping was performed only when the STIC value was less than 1.2 units. Subsequently, for the next 742 specimens, phenotyping was performed on all sera, irrespective of the STIC value. Table 1 shows that approximately half of the MS Pi-types were missed initially by not typing all sera; 4.9 percent MS types appeared with STIC values less than 1.2 units, whereas 10.1 percent actually were present when all sera were typed. In contrast, the percentage of MZ and ZZ Pi-types was almost identical in the two phases of the study. Thus, for statistical analysis, the two studies can be combined for evaluating the prevalence of MZ, SZ, ZZ and SS Pi-types, but not for MS.

Table 1 shows that the MS prevalence was not significantly increased in 965 COPD patients as compared to our earlier control study, where heterozygous Z types (MZ mostly plus SZ) were significantly increased in the 965 COPD patients; 8.0 vs 2.8 percent in the control group (p<.0005). The ZZ severely deficient Pi-type was also significantly increased in number with 1.9 percent of the 965 patients with this type as compared to an estimated 0.04 percent in control studies performed elsewhere; our control study of 1,380 subjects did not contain any with a ZZ type. Three patients with an SS Pi-type had intermediate AAT deficiency and also contributed to the total number of intermediate deficient Pi-types.

Age of Patients

The total group of COPD patients had a mean age of 65.3 ± 7.5 years, ranging between 41 and 86 years. Neither the MS nor the MZ patients differed significantly in age from the group as a whole or from a subgroup of patients with an MM phenotype (64 ± 8.7 yrs, n=106). Those patients with a homozygous ZZ phenotype, however, tended to be younger with a significantly lower mean age of 55.9 ± 9.8 yrs (Table 2).

STIC values for Pi-types

Table 3 lists the STIC values for the four major Pi-types and the few less frequently encountered Pi-types found in our COPD patients. The STIC values for almost all Pi-types are higher than in our previous investigations; this is most likely due to the severe illness and stress that the patients in our current study were experiencing. The values obtained in our 1972 study are included in Table 3 for comparison. Only two of the MZ Pi-types had STIC values above 1.2 units and would have been missed had Pi-typing been limited.
Table 3—STIC Values for Various Pi-types in COPD Patients

<table>
<thead>
<tr>
<th>Pi-Type</th>
<th>n</th>
<th>STIC</th>
<th>STIC, 1972 Study*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(Mean ± SD)</td>
<td>(Mean ± SD)</td>
</tr>
<tr>
<td>MM</td>
<td>132</td>
<td>1.585 ± 0.381</td>
<td>1.194 ± 0.244</td>
</tr>
<tr>
<td>MS</td>
<td>75</td>
<td>1.376 ± 0.338</td>
<td>0.970 ± 0.170</td>
</tr>
<tr>
<td>MZ</td>
<td>74</td>
<td>0.953 ± 0.167</td>
<td>0.730 ± 0.141</td>
</tr>
<tr>
<td>ZZ</td>
<td>18</td>
<td>0.182 ± 0.114</td>
<td>0.192 ± 0.051</td>
</tr>
<tr>
<td>SS</td>
<td>3</td>
<td>0.755 ± 0.069</td>
<td>0.682 ± 0.110</td>
</tr>
<tr>
<td>SZ</td>
<td>2</td>
<td>0.676 ± 0.065</td>
<td>0.504 ± 0.098</td>
</tr>
<tr>
<td>IZ</td>
<td>1</td>
<td>0.589</td>
<td></td>
</tr>
<tr>
<td>GS</td>
<td>1</td>
<td>1.074</td>
<td></td>
</tr>
<tr>
<td>GM</td>
<td>3</td>
<td>1.302 ± 0.181</td>
<td></td>
</tr>
<tr>
<td>IM</td>
<td>2</td>
<td>1.460 ± 0.262</td>
<td>0.950</td>
</tr>
<tr>
<td>MP</td>
<td>1</td>
<td>1.425</td>
<td>0.987 ± 0.235</td>
</tr>
</tbody>
</table>

*This control study was performed on subjects much younger than our patients with COPD. It included 1,380 seventh grade junior high school students aged 12-14 yrs old. However, AAT phenotypes and STIC levels are not known to change with age, except that subjects with severe AAT deficiency may be lost from older-age groups.

only to those with an STIC of less than 1.2 units.

**Discussion**

This study of AAT phenotypes in 965 patients with COPD confirms our 1969 study which found an increased prevalence of intermediate AAT deficiency. The majority of the deficient patients have the MZ Pi-type, with a few having an SZ or SS type. Approximately 8.3 percent of patients in the present study had deficient Pi-types in contrast to a prevalence of 15.2 percent with intermediate deficient STIC levels in the older study. The somewhat lower percentage currently could result from the older mean age of the patients, and their broader category of COPD compared to the specific diagnosis of emphysema studied previously.

The detection of an increased prevalence of intermediate deficient (usually heterozygous) phenotypes in COPD populations has been noted by several other investigators. However, a second approach for evaluating intermediate AAT deficiency in causing COPD had negative results, in that abnormal pulmonary function was not found in subjects with intermediate AAT deficiency in general populations. Other investigators sought an answer by determining whether MZ subjects, smokers or nonsmokers, had an increased rate of lung deterioration over time. Ostrow and Cherniak found that deterioration of lung elasticity was accelerated in the intermediate deficient state. It is generally agreed that the increased risk of developing COPD in a person with intermediate AAT deficiency is much lower than that of a ZZ homozygote, so that most heterozygotes detected in a screening program will indeed have normal lung function; of importance, too, is the likelihood that those heterozygotes with lung disease frequently will not be included in studies of healthy populations.

Thus, the fairly consistent finding of an increased percentage of COPD patients with intermediate AAT deficiency, especially the MZ Pi-type, comprises the strongest obtainable evidence of a role for intermediate AAT deficiency in pathogenesis of COPD.

It is clear from available data that the percentage of patients with COPD and a predisposing abnormality of AAT is small (10-25 percent). However, recent studies suggest that an acquired relative deficiency of AAT may occur more commonly than the genetic deficiency, since cigarette smoking appears to reduce the effective level of AAT in the blood and to increase the amount of elastolytic protease in polymorphonuclear leukocytes and macrophages, in effect mimicking the genetic intermediate deficiency state. The significance of the ZZ Pi-type in predisposing to COPD is unquestioned, yet it accounts for only 2-8 percent of cases.

The overall mean age of COPD patients in this study is older than in our previous study (65 vs 54 years). However, similar to our previous study, heterozygotes did not differ in age from the overall group, whereas severely deficient homozygotes were significantly younger. Previously, the age of onset of respiratory symptoms, however, was younger in both homozygotes and heterozygotes, whereas such data were not available in the current study.

STIC values were higher in the current study for all Pi-types of COPD patients than in our previous study. It is believed that this is due to the more severe illness and stress that these patients were undergoing as compared to our previous study. In 1969, only non-hospitalized male veterans were being studied, whereas severely ill patients, including a number of post-menopausal women who may have been receiving estrogens, were studied currently. AAT is an acute phase reactant protein so that the serum level rises with acute illness, stress, or administration of estrogen. Most AAT variants respond to these factors with a rise in serum level, whereas the Z variant cannot respond.

We conclude from this study of 965 COPD patients that there is an increase in the total of Pi-types that produce intermediate degrees of AAT deficiency, especially MZ. These data are the most direct evidence to support the concept that an intermediate deficiency state of AAT makes one prone to develop COPD. The deficiency, as shown in previous studies, enhances the detrimental effect of cigarette smoking for inducing lung destruction.

**References**

2. Lieberman J. Heterozygous and homozygous alpha-antitrypsin

Alpha-Antitrypsin Pi-Types in COPD Patients (Lieberman, Winter, Sastre)
6 Lieberman J. This week's citation classic. Current Contents 1983; 38:23