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Alpha-1-antitrypsin levels and prevalence of Pi variant phenotypes in adult Nigerian asthmatics

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Summary

Alpha-1-antitrypsin (A1AT) phenotypes and serum levels were determined in 99 asthmatic patients and 100 control subjects. The phenotypes encountered were PiMM 74% in asthmatics, 98% in controls; PiMZ 19% in asthmatics, 1% in controls; PiMW 3% in asthmatics, 0% in controls; and PiLM 2% in asthmatics, 1% in controls. There was one asthmatic patient with the homozygous deficient phenotype ZZ. The result revealed that there were more deficient heterozygous phenotypes in the asthmatic group than the control group. There was also a positive correlation between the number of patients with deficient phenotypes and the severity of asthma ($P < 0.02$). Analysis of the serum A1AT levels revealed that as a group the asthmatic patients had significantly lower A1AT levels (1.97 ± 0.18 g/l) than the control group (2.21 ± 0.15 g/l) ($P < 0.01$). However, there was no statistically significant difference in the A1AT serum levels of patients with PIMM phenotype and the control of the same phenotype. Statistical analysis could not be done for the other phenotypes because of the small number of subjects. Apart from the patient with PiZZ the A1AT serum levels encountered in the study were not low enough to justify replacement therapy with alpha-1-proteinase inhibitor in our asthmatic patients.

Résumé

Les phénotypes alpha-1-antitrypsin (A1AT) et les niveaux de sérum furent déterminés chez 99 patients asthmatiques et 100 sujets de contrôle. Les phénotypes rencontrés furent PiMM 74% chez les asthmatiques, 98% chez les contrôles; PiMZ 19% chez les asthmatiques, 1% chez les contrôles; PiMW 3% chez les asthmatiques, 0% chez les contrôles; PiLM 2% chez les asthmatiques, 1% chez les contrôles. Un parmi les patients asthmatiques fut de phénotype homozygote déficient ZZ. Ce résultat révèle qu'il y eut plus de phénotypes homozygotes déficients chez les asthmatiques que chez le groupe de contrôle. Il y eut aussi une corrélation positive entre le nombre de patients de phénotypes déficients et la sévérité de l'asthme ($P < 0.02$). L'analyse du niveau de sérum A1AT révéla que le groupe de patients asthmatiques avait un niveau de A1AT significativement plus bas (1.97 ± 0.18 g/l) que le groupe de contrôle (2.21 ± 0.15 g/l) ($P < 0.01$). Cependant, il n'eut pas de différence statistiquement significative entre les niveaux de sérum A1AT des patients de phénotype PiMM et les sujets de contrôle du même phénotype. L'analyse statistique n'a pas pu être faite pour les autres phénotypes à cause du petit nombre des sujets. En dehors des patients avec PiZZ, les niveaux de sérum A1AT rencontrés dans cette étude ne furent pas suffisamment bas à pouvoir justifier un remplacement thérapeutique avec l'inhibiteur de l'alpha-1-proteinase chez nos patients asthmatiques.

Introduction

Alpha-1-antitrypsin (A1AT) is one of the anti-inflammatory host defence proteins of the respiratory tract. It is synthesized in the liver and inhibits the proteolytic actions of trypsin, plasmin and elastase [1]. Its deficiency state has been well studied for the Z allele which is associated with early onset of emphysema [2] as well as liver cirrhosis [3]. At present, about 30

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different alleles of A1AT have so far been identified [4]. These are combined in 46 different phenotypes, seven homozygous and 39 heterozygous, constituting the Pi system [5]. Apart from the Z allele, other deficient alleles are Pil, P, S, W and null (—) which are associated with reduced serum A1AT levels [6–8]. More recently, some variants of the M phenotype, M Malton, M Duarte, M Lamb and M Baldwin have been found to produce grossly reduced levels of A1AT with risks of developing emphysema in both heterozygotes and homozygotes [9, 10]. The literature review on A1AT and asthma suggests that there may be an association between heterozygote-deficient phenotypes and severe asthma [6, 11]. However, other workers [12, 13] did not find any strong association between variant phenotypes such as PiMZ or PiMS and severity of asthma. It is known that during inflammation, the A1AT levels in patients with heterozygote-deficient phenotypes do not rise as high as levels of PiMM patients [13]. If this statement is true, it is possible that these deficient phenotypes may be important in patients with severe asthma which is marked by recurrent inflammatory process [14].

This study was designed to find out whether patients with severe asthma have a higher prevalence of deficient A1AT phenotypes than those with less severe asthma. It was also to determine whether the serum A1AT concentration in those patients with heterozygous-deficient phenotype was low enough to justify replacement therapy with alpha-1-antiproteinase inhibitor as was recently advocated for emphysematous patients with A1AT deficiency [15].

Subjects and methods

Consecutive patients with bronchial asthma attending the chest clinic of the University College Hospital, Ibadan, were studied.

Diagnosis of asthma was according to the standards of the American Thoracic Society [16] while the Jones Index [17] was used for classifying the severity of the asthma into mild, moderate and severe disease. Other clinical data such as age, sex, height and smoking status were recorded. Investigations carried out included standard posterior–anterior chest radiograph and spirometry. This involves using the vitalograph dry wedge spirometer to measure the forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC). The values obtained after matching for age, sex and height and correcting for racial differences were calculated as percentages of normal [18].

One hundred normal control subjects who were non-smokers and without any known respiratory or liver problems were also studied. Ten millilitres of blood were withdrawn from each patient. This was separated and the serum frozen and stored at − 20°C. Sodium azide was used as preservation to prevent bacterial degradation of the samples. The blood samples were collected over a 3-month period and kept in an ice-packed flask during transportation to the U.K. for analysis of A1AT phenotype. The A1AT serum level was determined by A.B.A.

Serum concentration of A1AT was measured by automated immunological methods described by Laurell [19]. The samples were diluted 1:50 and applied in 4-mm wells. The electrophoretic run was carried out for 5.5 h at 35 V/cm. A1AT concentration was expressed as a percentage of a normal pool where the pool was that of 1000 normal donor samples in the laboratory.

A1AT phenotype was carried out by isoelectric focusing in polyacrylamide gel (PIEF) using the method of Cox [4], a modification of techniques described by LKB, Stockholm, Sweden (supplier of the ampholytes). Gels 1 mm thick were cast using a gel solution containing 5% acrylamide of which 2.5% was bisacrylamide, 9.5% sucrose, 0.021% ammonium persulphate, 0.055% TEMED and 5% ampholyte. About 20 µl of serum were supplied using LKB application strips. In some runs, serum was reduced by pre-incubation with dithloerythritol (DTE), at a final concentration of 10 mM. The run was carried out on an LKB multiphor apparatus with an LKB 2301 power supply. Prefocusing was usually carried out for 30 min and the total length of the run after sample application was 3 h, temperature 10–15°C. Prepared LKB plates, pH 4–5, were also used. Staining with coomassie blue B250 was carried out as described in the LKB literature but using a reduced strength of stain (0.05% in 3 M acetic acid:methanol:water; 3:9:3, v/v). Destaining was carried out for 1 h in a 1:1 mixture of solvents and 5% acetic acid. In some

cases, immunofixation was carried out after PiEF. Samples were diluted to contain approximately 0.022 mg A1AT/ml. Antiserum diluted 2:1 was applied directly to the surface of the gel. The gel was washed for 1–3 days in saline, then stained as outlined above.

Statistical comparisons between the various groups were made using Student's *t*-test and the chi-square test.

Results

Only 99 out of 115 asthmatic patients and the 100 normal subjects serving as controls were analysed. This was because some of the sera were already degraded during transport to Sheffield. The age and sex distribution of the subjects is as shown in Fig. 1.

The A1AT phenotype profile is shown in Table 1. The majority of the asthmatic patients (74%) and 98% of the control had PiMM, which is the normal phenotype. The heterozygote phenotypes encountered were PiMZ in 19% asthmatics and 0% controls. There was one asthmatic patient with the homozygous-deficient phenotype PiZZ. When the asthmatics and control groups were compared, there were more deficient phenotypes in the asthmatic group than the control ($P < 0.02$). Table 2 shows the distribution of the various phenotypes in relation to severity of asthma. If the number of patients with heterozygote-deficient phenotypes in the moderate and severe asthmatics group are taken together and compared with those with mild asthma, there is a statistically significant difference between the two groups ($P < 0.01$). It is noteworthy that the homozygote-deficient PiZZ was considered to have moderately severe asthma. This particular patient was a 34-year-old male.

The results of the serum A1AT levels (Table 1) showed that as a group the asthmatic patients had significantly lower values (mean ± s.e.m. 1.97 ± 0.18 g/l) than the control group (2.21 ± 0.15 g/l) ($P < 0.01$). There was no statistical difference in the A1AT serum levels of the asthmatic patients with PiMM and controls of the same phenotype. The statistical significance of the other phenotypes could not be ascertained because of the small number of subjects. Apart from the patient with homozygous-deficient phenotype who had an A1AT level of 0.58 g/l all other patients had values within the normal range of 1.4–2.7 g/l.

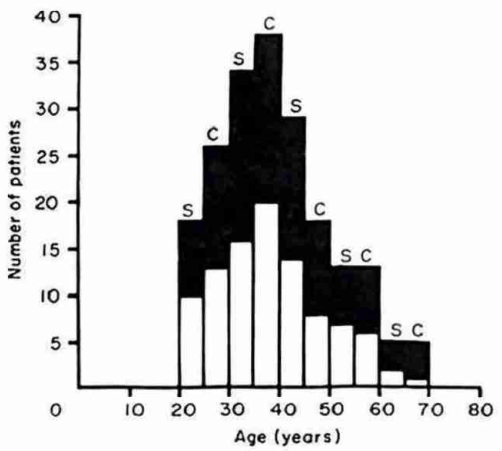

Fig. 1. Histogram showing age and sex (■, male; □, female) distribution of patients with asthma (S) and control subjects (C).

Discussion

There is a longstanding controversy regarding the prevalence and clinical significance of heterozygous-deficient phenotypes in asthma. Fagerhol and Hauge [6] reported a high proportion of PiMS in their asthmatic patients, an observation which was also made by Arnaud *et al.* [20]. The significance of these phenotypes in clinical practice was, however, not clear. Other workers [13, 21, 22], however, failed to observe any increased incidence of asthma in subjects with heterozygous-deficient phenotypes. We have proposed that if there was a relationship between A1AT variant phenotypes and asthma, there would be a high proportion of these phenotypes in our asthmatic patients. This was proved in our study as we observed a significant increased distribution of deficient phenotypes MZ, LM, MW and ZZ in our asthmatic patients than in control subjects. Furthermore, we were also able to show that patients with moderately severe and severe asthma had more deficient phenotypes than patients with mild asthma. It is recognized that during stress and inflammation, A1AT levels in individuals with heterozygous phenotypes do

Table 1. Profile of A1AT phenotypes and serum concentration in asthmatic patients and normal control

A1AT phenotypes	No. of patients with asthma	Mean A1AT concentration (g/l)	No. of control subjects	Mean A1AT concentration (g/l)	*P* value
MM	74	2.11 ± 0.12	98	2.25 ± 0.39	>0.05
MZ	19	1.68 ± 0.15	1	2.1	—
ZZ	1	0.58	—	—	—
LM	2	1.83	1	1.9	—
MW	3	1.75 ± 0.15	—	—	—

Table 2. Distribution of A1AT phenotypes in relation to severity of asthma

A1AT phenotypes	Mild asthma	Moderate asthma	Severe asthma	Total
MM	34	23	17	74
MZ	3	10	6	19
MW	1	2	—	3
LM	—	1	1	2
ZZ	—	1	—	1

Key to severity: mild asthma, $FEV_1 > 70\%$ of predicted value; moderate, 40–70% of predicted value; severe, < 40% of predicted value.

not rise as much as those in individuals with PiMM [13]. This will thereby make the anti-inflammatory defence function of such patients defective.

Also worthy of note is that the homozygous-deficient phenotype PiZZ was encountered for the first time in this environment in our study. This was in a patient with moderately severe asthma without any clinical evidence of emphysema. The other heterozygous-deficient phenotypes observed in our study include the PiLM and PiMW. These are new phenotypes in this environment as previous studies did not reveal them [23, 24]. This may be due to the facts that these studies were in normal individuals and that the number of subjects studied was rather small. Furthermore, our technique for determining phenotypes was different from theirs. The first observation in Africa of the LM phenotype was in Zaire; it is believed to have migrated from India where it was first encountered [25]. It is believed that when larger numbers of subjects are screened it is possible for more abnormal phenotypes to be discovered than the present few.

Our results of A1AT serum level concentrations were, however, inconclusive. Apart from the homozygous-deficient patient ZZ who had very low A1AT concentration all the other patients had values within the normal range of 1.4–2.7 g/l. The asthmatic patients as a group had significantly lower A1AT levels than the control group which is similar to the observation of Aderele *et al.* in the same environment [26]. However, we failed to detect any significant differences in A1AT levels between the asthmatic patients and the control groups in respect of the different phenotypes. It is, however, possible that if the A1AT serum levels were monitored during steady state and periods of acute exacerbation it may be possible to detect differences amongst the various phenotypes. It is therefore likely that the difference in A1AT values between the asthmatic patients and the controls was a result of the inherent low serum concentrations in those patients with deficient phenotypes.

An extension of this study will be to monitor the A1AT levels in asthmatics during periods of exacerbation and remission. It may then be

possible to detect differences amongst the various phenotypes.

In conclusion, we have succeeded in establishing a relationship between the severity of asthma and the presence of variant-deficient A1AT phenotypes. We have observed the presence of the homozygous-deficient phenotype in one of our asthmatic patients. Furthermore, we found that the A1AT serum levels were significantly lower in the asthmatics as a group than the control group. However, the A1AT levels encountered were not low enough to advocate replacement therapy with A1AT protease inhibitor.

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