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Serum alpha$_1$-antitrypsin levels in asthmatic children

W. I. ADERELE*, C. OJO, V. O. OSANYINTUYI AND O. ODUWOLE
Department of Paediatrics, University College Hospital, Ibadan, Nigeria

Summary

Serum levels of alpha$_1$-antitrypsin (AAT) were determined by an enzymatic assay method in fifty-five asthmatic children and in the same number of controls. The mean AAT level was significantly lower in asthmatics (1.65 μmol/min/ml) than in controls (2.0 μmol/min/ml) ($P < 0.02$). A significantly higher proportion of asthmatics than controls ($P < 0.05$) had levels below 2.1 μmol/min/ml which is the lower limit of normal, thus suggesting a higher prevalence of partial (heterozygous) AAT deficiency in the asthmatics. There was no relationship between the mean AAT levels and age, duration of asthma or frequency of asthmatic attacks. Although there is some controversy about the relationship between heterozygous AAT deficiency and pulmonary disease, severe (homozygous) AAT deficiency has been linked with emphysema which is also a complication of asthma. There was however, no evidence of emphysema in either the asthmatic child or the control who had no detectable serum AAT. There were three asthmatics whose chest radiographs showed hyperinflation, but had a mean AAT level that was not significantly different from that in those without such changes. Further studies, including phenotype determination in a larger group of asthmatic children, are required in order to determine the prevalence of both homozygous and heterozygous AAT deficiencies which may be risk factors in the development of emphysema and other pulmonary complications of asthma.

Résumé

Les taux de l'enzyme alpha-1-antitrypsine (AAT) à partir du serum ont été determinés utilisant la méthode d'essai enzymatique chez cinquante-cinq enfants souffrant de l'asthme et chez la même nombre des témoins. Les valeurs moyennes du taux d'AAT était significativement plus basse dans les cas asthmatiques (1.65 μmol/min/ml) que chez les témoins (2.0 μmol/min/ml) ($P < 0.02$). Une proportion significantivement plus haute chez les cas asthmatiques que les témoins ($P < 0.05$) ont les taux moins de 2.1 μmol/min/ml qui est la limite basse du normal. Ceci suggère une fréquence plus haute de carence partielle (hétérozygote) chez les enfants asthmatiques. Il n'y avait pas de relation entre les taux moyens d'AAT et l'age, la durée de l'asthma ou la fréquence des asthmatiques. Bien qu'il y a quelque controverse sur la relation entre la carence d'AAT hétérozygote et la maladie pulmonaire, la carence d'AAT (homozygote) a été lié avec l'emphysème pulmonaire qui est aussi une complication de l'asthme. Cependant il n'avait pas de preuve certaine de l'emphysème pulmonaire chez l'enfant asthmatiques ou chez le témoin qui n'avait pas eu l'AAT sérique découverte. Il y'avait trois enfants asthmatiques qui ont démontré l'hypergonflement sur la radiographie de poitrine, mais qui ont le taux moyen d'AAT pas significativement different que ceux sans tels changements. Les études supplémentaires, inclurant la determination phénotypique chez un groupe plus large d'enfants asthmatiques est nécessaire pour q'on puisse déterminer la fréquence d'homozygote et d'hétérozygote des carences AAT homozygote et héterozygote qui peut-être les facteurs risques dans l'évolution de l'emphysème et l'autres complications pulmonaires de l'asthme.

*To whom correspondence should be addressed.

Introduction

Alpha$_1$-antitrypsin (AAT), a glycoprotein enzyme which is synthesized in the liver, inhibits the proteolytic actions of trypsin, plasmin and elastase (Schwartz, 1980). A severe deficiency of this enzyme has been associated with liver disease in children (Glasgow *et al.*, 1973; Talamo, 1975) as well as in adults (Berg & Ericksson, 1972; Ayoola, 1982). Several cases of emphysema in adults (Laurell & Ericksson, 1963; Ericksson, 1965; Talamo, Blennerhassett & Austen, 1966; Lieberman, 1969) and a few in children (Glasgow *et al.*, 1973; Talamo, 1977) have also been attributed to this enzyme deficiency. Although bronchial asthma does not normally progress to emphysema, a small proportion of asthmatics with chronic severe disease develops emphysema at some stage of their disease (Pearlman & Bierman, 1980). According to Pearlman and Bierman (1980), this development is usually due to the chronic, partial obstruction of the bronchus by mucus plugging, although there are speculations that tracheo-bronchial ciliary dysfunction and smooth muscle hyperplasia may also play some part.

There have been reports of an association between partially deficient (heterozygous) AAT variants in adults and children and increased liability to chronic respiratory diseases including asthma (Fagerhol & Hague, 1969; Arnaud *et al.*, 1976; Katz, Lieberman & Siegel, 1976). This association would suggest a deficiency of AAT as a predisposing factor to the development of emphysema in some asthmatic patients. The purpose of the present study was to estimate the serum levels of AAT in asthmatic children and controls in order to determine if there is a higher prevalence of the partial or total deficiency of this enzyme in the asthmatics. We have also examined a possible relationship between the serum levels of AAT and the frequency of asthmatic attacks.

Materials and methods

The subjects were fifty-five asthmatic children (thirty-one males, twenty-four females), aged between 2 and 16 years, whose asthma had lasted between 1 and 15 years (mean, 5.9 years). The diagnostic criteria for asthma were as earlier reported by Aderele and Oduwole (1983). The severity of asthma was based on the frequency of acute attacks during the previous 12 months. The disease was graded as mild in patients who had one acute attack or none per month, while in those who had more than one attack per month over the same period, it was graded as severe asthma. None of the subjects had an overt attack of asthma at the time blood specimens were obtained for the estimation of the enzyme level, but eleven (20%) of the fifty-five had persistent bronchial obstruction manifested by the presence of respiratory rhonchi. All the patients were on bronchodilator therapy consisting of Ventolin, Franol, and Tedral, taken only when needed, but none had received steroid therapy within 3 months of the study. Standard chest radiographs were taken during asymptomatic periods in forty-eight of the fifty-five patients within 3 months of the study.

There was no history suggestive of liver disease in any of the patients, neither was any significant degree of hepatomegaly present except in one child whose liver was palpable, 5 cm below the costal margin. The liver function tests were normal in all the patients as well as in the controls who were apparently healthy children with no known family or personal history of respiratory or hepatic disease. The controls were matched for sex, age and socio-economic background with the subjects. Informed consent was obtained from the parents of both subjects and controls.

Three to 5 ml of venous blood was withdrawn from each subject or control. The blood was centrifuged within 1 h of collection. The sera thus obtained, was kept in a freezer at −20°C until analysed. The levels of alpha$_1$-antitrypsin activity was determined by an enzymatic assay method described by Dietz, Runbinstein and Hodges (1974). Statistical analysis was by means of the Student's t and chi-square tests.

Results

The mean age of the asthmatics was 8.3 years (s.d., 3.4) compared with 7.9 years (s.d. 3.5) in the controls ($P > 0.05$).

Alpha$_1$-antitrypsin levels

According to the method used in quantifying AAT serum levels in the study, 2.1 μmol/min/ml was the lower limit of normal levels (Dietz, Rubinstein & Hodges, 1974). The serum level of the enzyme was below this lower level of normal in forty-four (80%) of the fifty-five subjects and in thirty-three (60%) of the controls ($P < 0.05$).

AAT activity was not detectable in one female asthmatic whose chest radiograph was normal and in one female control. In three each, of the remaining asthmatics and controls, AAT levels were 0.8 μmol/min/ml and below.

The frequency distribution of serum AAT levels in both asthmatics and controls (Fig. 1) shows that the largest number of asthmatics and controls had AAT levels between 1.7 and 2.0 μmol/min/ml.

As shown in Table 1, the mean AAT level (2.01 μmol/min/ml) was significantly higher ($P < 0.02$) in male controls than in the male asthmatics (1.55 μmol/min/ml). Similarly, the mean level of the enzyme was higher in all the controls than in asthmatics ($P < 0.02$). The mean level of 1.98 μmol/min/ml in the female controls was however, not significantly different from 1.75 μmol/min/ml in the female asthmatics. There was no correlation between age and AAT levels ($r = 0.316$ in asthmatics and 0.302 in controls), neither was there any relationship between the sex and the mean levels in both asthmatics and controls.

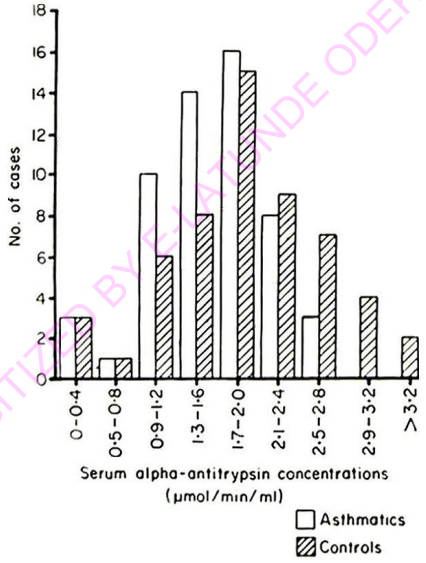

Fig. 1. Frequency distribution of serum alpha$_1$-antitrypsin concentrations in asthmatics and controls.

AAT and severity of asthma

The mean AAT levels were similar in the thirty-three with mild asthma and the remaining twenty-two with severe disease (Table 2).

AAT and chest radiographic changes

Chest radiographs were available in forty-eight (87%) of the fifty-five asthmatics. The highest mean value of 1.91 μmol/min/ml was found among the seventeen patients in whom there were radiographic inflammatory changes and this was significantly different ($P < 0.05$) from a mean of 1.55 μmol/min/ml in twenty-eight with normal X-ray (Table 3). The levels in the remaining three cases with changes of hyperinflation were 0.37, 1.46 and 2.5 (mean, 1.44) μmol/min/ml, respectively.

AAT and other variables in asthmatics

As shown in Table 4, there was no relationship between the mean AAT levels and (a) family history of asthma ($P > 0.5$); (b) the duration of asthma ($P > 0.1$); (c) the interval between the last acute attack of asthma and the day blood was obtained in forty-seven asthmatics from whom reliable information was obtained ($P > 0.5$); (d) the presence or absence of abnormal chest signs, mainly rhonchi, on clinical examination ($P > 0.5$); and (e) reactions to skin sensitivity test ($P > 0.5$).

Discussion

The present study has shown that, in addition to a significantly lower mean AAT level in asthmatics than in controls, the proportion of asthmatics with low AAT levels was significantly higher than that of the controls. This would suggest that there was probably, a higher

Table 1. Mean alpha$_1$-antitrypsin levels in asthmatics and controls

	Asthmatics*			Controls†			P
	No. of cases	Mean (μmol/min/ml)	s.d.	No. of cases	Mean (μmol/min/ml)	s.d.	
Males	31	1.55 (0.16–2.39)	0.51	31	2.01 (0.23–4.55)	0.84	< 0.02
Females	24	1.75 (0–2.75)	0.65	24	1.98 (0–3.37)	0.82	> 0.1
Total	55	1.65	0.59	55	2.00	0.82	< 0.02

Figures in parentheses: range of levels.
s.d. = Standard deviation.
*For asthmatics; males: females, $P > 0.1$.
†For controls; males: females, $P > 0.5$.

Table 2. Alpha$_1$-antitrypsin levels in relation to severity of asthma

	Mild cases	Severe cases
No. of cases	33	22
Mean (μmol/min/ml)	1.64	1.64
Range	0–2.75	0.37–2.5
Standard deviation	0.65	0.46
P	> 0.5	

Table 3. Mean alpha$_1$-antitrypsin levels and chest X-ray findings

Finding	No. of cases	Mean (μmol/min/ml)	Standard deviation	P
(a) Normal	28	1.55	0.51	*
(b) Inflammatory changes	17	1.91	0.47	*
(c) Hyperinflation	3	1.44	1.07	*

*a:b, $P < 0.05$; a:c; $P > 0.5$; b:c; $P > 0.1$.

incidence of partial (heterozygous) AAT deficiency in asthmatics than in controls.

Various workers have reported an association between heterozygous AAT deficiency and pulmonary disease including asthma, in childhood. Fagerhol and Hague (1969) reported an increased prevalence of asthma in individuals with intermediate AAT deficiency than in those with normal AAT phenotype. Similarly, Arnaud *et al.*, (1976) have reported a significantly increased frequency of deficient AAT phenotypes in an asthmatic population when compared with controls. The disease was also more severe in the deficient group. By contrast, other workers (Webb *et al.*, 1973; Schwartz *et al.*, 1973; Szczeklik *et al.*, 1974; Katz *et al.*, 1976; Schwartz *et al.*, 1977; Talamo, 1977) have reported that there is no increased incidence of asthma or other pulmonary disease in heterozygous individuals when compared with

Table 4. Alpha$_1$-antitrypsin levels in relation to some variables in asthmatics

Variable	No. of cases	Mean (μmol/min/ml)	Standard deviation	*P*
Family history of asthma:				
Positive	27	1.67	0.55	
				> 0.5
Negative	28	1.61	0.61	
Duration of asthma:				
0–5 yrs	28	1.71	0.58	
				> 0.1
> 5 yrs	27	1.56	0.58	
Last attack before study:				
0–7 days	18	1.62	0.65	
				> 0.5
> 7 days	29	1.72	0.51	
Abnormal chest signs:				
Absent	44	1.64	0.62	
				> 0.5
Present	11	1.62	0.36	
Skin sensitivity test:				
Positive	21	1.63	0.56	
				> 0.5
Negative	11	1.57	0.72	

those with normal phenotype. In the present study, there was an increased incidence of low AAT levels in the asthmatics when compared with the controls, but there was no relationship between the severity of asthma or its duration and the AAT levels.

Although several cases of emphysema with onset during early adulthood have been reported in patients with homozygous AAT deficiency (Eriksson, 1965; Talamo, Blennerhassett & Austen, 1966; Guenter *et al.*, 1968; Lieberman, 1969), reports of similar development during childhood are few (Talamo *et al.*, 1971; Glasgow *et al.*, 1973 Talamo, 1977). There is also a paucity of information about the role of homozygous AAT deficiency in childhood asthma. The present study has not suggested any association between severe AAT deficiency and pulmonary radiographic changes. Although one of the three asthmatic children in the present study with radiographic changes of hyperinflation which might suggest a degree of emphysema had a very low AAT level in the range that is usually associated with homozygous deficiency, the mean AAT in the three did not differ significantly from the mean levels in the other asthmatics. Besides, one asthmatic child with a normal chest radiograph and one of the controls also had undetectable AAT level.

The quantitative enzymatic assay method used in the present study is precise and rapid in execution. However, it suffers from the fact that it estimates the total serum antitryptic activity and not the alpha$_1$-antitrypsin alone (Dietz *et al.*, 1974). Yet, a small proportion of the antitrypsin capacity of serum is attributable to other antitryptic proteins, primarily the alpha$_2$-antitrypsin (Dietz *et al.*, 1974). Thus, the levels obtained in the present study were probably higher than the true AAT levels. In normal subjects, any acute or chronic inflammatory disorder usually results in an increase in serum alpha$_1$-antitryptic activity (Dietz *et al.*, 1974). This phenomenon might therefore, have accounted for the significantly higher AAT levels in asthmatics with radiographic changes of inflammation than those with normal films. It is however, conceded that some of these inflammatory changes were

probably areas of collapse rather than areas of inflammatory consolidation. In view of the fact that inflammation and other factors may distort the true serum AAT levels, the use of serum levels to detect individuals with mild to moderate AAT deficiency is not totally reliable. The determination of the phenotypes has therefore, been suggested as being more reliable than serum assays, in separating homozygous deficient cases from those with heterozygous or intermediate deficiency and others with normal phenotype (Breit & Penny, 1980).

If the results in the present study, suggesting a higher proportion of asthmatics with heterozygous AAT deficiency than controls were confirmed by phenotype determination in larger series, then it may be concluded that there is a significant proportion of asthmatic children that are at risk of developing other respiratory disease including emphysema. This risk will be in addition to that which they already run in the development of the latter complication due to prolonged partial obstruction of the bronchi by mucus plugging. A high prevalance of AAT deficiency would suggest that routine serum AAT assays and phenotype determinations should be undertaken in asthmatic children in order to detect early those who might be at risk of developing emphysema in early adulthood, and to monitor them more closely during follow-up. Such patients would also be strongly advised against indulging in activities such as cigarrete smoking that might hasten the onset of this and other respiratory complications of AAT deficiency and asthma.

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