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Alpha-1-antitrypsin and chronic bronchitis in adult Nigerians

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Summary

Alpha-1-antitrypsin (A1AT) serum levels and phenotypes were determined in 49 Nigerians with chronic bronchitis and 100 normal controls. The A1AT phenotypes encountered were PiMM, 69% in chronic bronchitics, 98% in controls; PiMZ, 23% in chronic bronchitics, 1% in controls; PiLM, 2% in chronic bronchitics, 1% in controls. There were three patients (6%) with the homozygous-deficient phenotype PiZZ. Spirometry confirmed obstructive ventilatory pattern in the patients with chronic bronchitis, and the difference in the values obtained between the patients and controls was statistically significant ($P < 0.01$). Serum A1AT levels were within the normal range of 1.4–2.7 g/l in all except the three patients with PiZZ. There was no significant difference in the A1AT serum levels between patients with chronic bronchitis and control subjects with the PiMM phenotype; tests of significance were not possible for the other phenotypes because of the small number of subjects. The observation of PiZZ in 6% of our patients with chronic bronchitis is in support of screening of this category of patients. Replacement therapy with alpha-1-antitrypsin inhibitor is currently under investigation and may be worthwhile in these patients if detected early.

Résumé

Les phénotypes et les niveaux de sérum alpha-1-antitrypsin (A1AT) furent déterminés chez 49 Nigérians avec la bronchite chronique et 100 contrôles normaux. Les phénotypes A1AT rencontrés furent PiMM, 69% chez les bronchitiques chroniques, 98% chez les contrôles; PiMZ, 23% chez les bronchitiques chroniques, 1% chez les contrôles; PiLM, 2% chez les bronchitiques chroniques, 1% chez les contrôles. Trois patients (6%) furent de phénotype homozygote déficient PiZZ. La 'spirométrie' confirma un modèle de ventilation obstructif chez les patients souffrant de bronchite chronique et la différence entre les valeurs obtenues entre les patients et les contrôles était statistiquement significative ($P < 0.01$). Les niveaux de sérum A1AT furent dans l'interval normal de 1,4–2,7 g/l chez tous sauf chez les trois patients avec PiZZ. Il n'y eut pas de différence significative en niveaux de sérum A1AT entre les patients souffrant de bronchite chronique et les sujets de contrôle de phénotype PiMM; alors qu'une analyse statistique ne fut pas possible pour les autres phénotypes a cause du petit nombre de sujets. L'observation de PiZZ chez 6% de nos patients souffrant de bronchite chronique soutient le triage de cette categorie de patients. La thérapeutique de remplacement avec l'inhibiteur de l'alpha-1-antitrypsin actuellement sous étude pourrait être utile chez ces patients s'ils soient dépistés tôt.

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Introduction

Alpha-1-antitrypsin (A1AT) is a human protein which inhibits several proteolytic enzymes, especially trypsin, and was first isolated in 1962 [1]. This enzyme is synthesized in the liver and it is therefore not a coincidence that its deficiency state will be well marked in the liver. A1AT is one of the most polymorphic of human proteins and is under the control of the alleles transmitted in the autosomal co-dominant mode [2]. At present, about 30 different alleles of A1AT have so far been identified [3]. These products are combined in 46 different phenotypes, seven homozygous and 39 heterozygous, constituting the Pi system [4].

The pathological manifestations of A1AT deficiency have been extensively studied for the Z allele. Some other deficient alleles Pi1, P, S, W and null (—) are also associated with reduced A1AT serum levels [5–7]. More recently, some variants of the M phenotypes 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 [8–11]. However, of all these, it is the homozygous-deficient Z allele that is best known and also extensively studied. It is classically associated with early onset of emphysema [12] and chronic liver disease [13].

The literature review of A1AT in this environment revealed that studies have been confined mainly to normal patients [14, 15], patients with liver disease [16], and children with asthma [17]. In none of these studies was the homozygote-deficient ZZ phenotype encountered.

The aim of this study is to determine the A1AT profile in our patients with chronic obstructive bronchitis and emphysema, a study which has not previously been carried out in this environment. It is expected that it is perhaps in this group of patients rather than the normal population that the deficient phenotypes may be discovered.

Subjects and methods

Consecutive patients seen at our chest clinic diagnosed as having chronic bronchitis according to the CIBA criteria [18] were studied. All the subjects had full clinical evaluation, and data such as age, height, sex and smoking status were recorded.

Investigations carried out included standard posterior–anterior chest radiographs and spirometric studies. 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 forced expiratory ratio (FER) $FEV_1/FVC \times 100$ was determined for each subject. The severity of the disease was determined using the criteria stated below [19].

Normal/mild chronic bronchitis FER = 60–70%
Moderately severe chronic bronchitis FER = 40–60%
Severe chronic bronchitis FER < 40%

Ten millilitres of blood were withdrawn from each subject; this was separated and the serum frozen and stored at −20°C. Sodium azide was used as preservative 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 transport to the U.K., for analysis of A1AT phenotype while the A1AT serum concentration was carried out by A.B.A. Serum concentration of A1AT was measured by the automated immunological methods described by Laurell [20]. 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 [21], 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% 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 preincubation with dithioerythritol (DTE), at a final concentration of 10 mM. The run was carried out on an LKB Multiphore 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 acetic acid:methanol:water; 3:9:9, 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.

Results

Only 49 out of 56 subjects with chronic bronchitis and 100 normal subjects serving as controls could be analysed at the end of the study. This is because some of the serum samples degraded during transportation to the U.K. where the analysis was done.

The age and sex distribution of the subjects is as shown in Fig. 1. This reveals that the majority of the subjects with chronic bronchitis were over 50 years of age and predominantly male. The three subjects with PiZZ phenotype were below 50 years (40, 43 and 44 years, respectively). All the patients with chronic bronchitis satisfied the CIBA criteria [18] for diagnosis of chronic bronchitis. They also had typical irreversible obstructive airways pattern on spirometry. Table 1 shows the result of the spirometric findings which confirms that all the subjects with chronic bronchitis had moderate and severe airway obstruction according to the criteria we used.

On the other hand, the control subjects had normal spirometry and the difference in the FER between the PiMM chronic bronchitics and controls was statistically significant ($P < 0.01$). Analysis in respect of the other groups was not carried out because of the small number of subjects. It is noteworthy that the three subjects with PiZZ had severe airways obstruction. The smoking pattern reveals that all the subjects with chronic bronchitis were smokers.

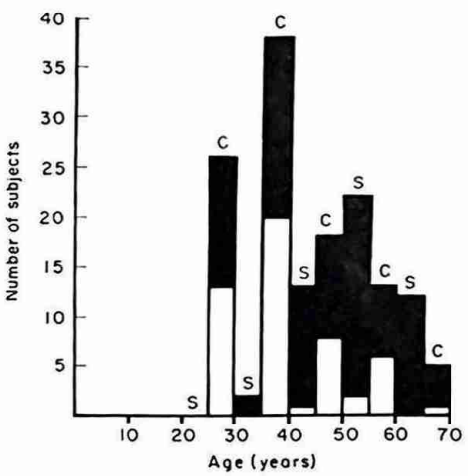

Fig. 1. Histogram showing age and sex (■ male, □ female) distribution of subjects with chronic bronchitis (S) and control subjects (C).

Table 2 shows the results of the profile of A1AT phenotypes and serum concentrations. The phenotypes encountered are PiMM 34 (69%) in chronic bronchitics, 98% in controls; PiMZ 11 (23%) in chronic bronchitics, 1% in controls, PiLM one (2%) in chronic bronchitics, 1% in controls. There were three (6%) subjects with the homozygous-deficient phenotype PiZZ. The serum levels of A1AT in respect of the various phenotypes is also shown in Table 2, while the subjects with the homozygous-deficient phenotypes had abnormally low levels (0.48 ± 0.21 g/l). Other subjects with chronic bronchitis and controls had levels within the normal range of 1.4–2.7 g/l. Furthermore, there were no significant differences in the A1AT serum levels of subjects with chronic bronchitis and control subjects with the PiMM phenotype ($P > 0.05$).

Table 3 is a comparison of the results from our study with those of other studies.

Discussion

The result from this study has confirmed the findings from most countries that PiMM is the commonest phenotype. However, of importance is the observance of the homozygote-deficient phenotype ZZ for the first time in this environment. The homozygote-deficient phenotype ZZ was, however, found only in our subjects with chronic bronchitis. Erikson [12] reported 50 cases of PiZZ out of which 30 had chronic bronchitis while the other 20 did not have pulmonary disease. This suggests that not all individuals with A1AT develop pulmonary disease.

The frequency of the ZZ phenotype has been estimated at 0.029% [7] although this was in a normal population screening. The levels of A1AT in this group of subjects are usually below 1.4 g/l. The pathogenesis of pulmonary lesions in A1AT deficiency is not well known. However, the hypothesis that because of the low levels of A1AT in these patients the lungs are not able to withstand the action of endoproteases, and therefore autodigestion occurs with consequent pathological changes [22], is probably still valid. Studies on homozygotes developing emphysema show no correlation

Table 1. A1AT phenotypes and mean serum concentration in subjects with chronic bronchitis and normal subjects

A1AT phenotypes	Chronic bronchitis (*n*)	Mean A1AT concentration of subjects with chronic bronchitis (g/l)	Control subjects (*n*)	Mean A1AT concentration in control subjects (g/l)	*P* value
MM	34	2.18 ± 0.21	98	2.25 ± 0.39	>0.01
MZ	11	1.7 ± 0.15	1	2.1	—
ZZ	3	0.48 ± 0.21	—	—	—
LM	1	1.8	1	1.9	—

Table 2. Correlation between spirometry (FEV_1/FVC = FER) and A1AT phenotypes in subjects with chronic bronchitis (CB) and control subjects (C)

A1AT phenotypes	Chronic bronchitis (*n*)	FER in subjects with chronic bronchitis (%)	Control subjects (*n*)	FER in control subjects (%)	*P* value
MM	34	58.15 ± 14.2	58	71.2 ± 11.5	>0.01
MZ	11	52.5 ± 10.5	1	75	—
ZZ	3	41.3 ± 12.5	—	—	—
LM	1	50	—	68.5	—

between severity of A1AT deficiency and severity of lung disease [23]. However, our three subjects with PiZZ had very low A1AT serum levels and severe airway obstruction, a pattern which is commonly described.

PiMZ was the next most common phenotype encountered in both subjects with chronic bronchitis and normal controls. The status of the MZ phenotype in the pathogenesis of chronic pulmonary disease is controversial. A number of reports have included observations of occasional A1AT-deficient heterozygotes with chronic pulmonary disease [24–26]. However, Welch *et al.* [23] in their study of patients with intermediate levels of A1AT concluded that intermediate A1AT levels are not important in pulmonary emphysema. Lieberman [27], in his study of patients with emphysema, found a sizeable proportion of the patients were heterozygotes although he was not able to advance a direct relationship. In this study, we also found that a sizeable number of our subjects with chronic bronchitis were heterozygotes. However, it was not possible to carry out other physiological tests, such as for gas transfer factor, that could have distinguished the patients with emphysema from those with only chronic bronchitis. It is also likely that the screening spirogram might not have uncovered a subtle disease that could be recognized only with more sensitive procedures such as measurement of residual volume, airway resistance, closing volume or pulmonary compliance at different rates of breathing. Thus the subjects with pure emphysema could not accurately be identified in this study. The smoking history obtained in our subjects was for clinical diagnosis of chronic bronchitis. Previous studies have shown conclusively a positive correlation between quantity of cigarettes smoked and severity of chronic bronchitis [28]. This present study was, however, not addressed to this.

It is noted that PiMS and SZ previously reported in this environment [14, 15] were not encountered in this study. The reason for the

Table 3. Pi phenotypes in different populations

Study	Population	Pi phenotypes							
		MM	MS	MZ	SS	SZ	ZZ	LM	MV
Fagerhol and Tenfford (1968) [31]	Spain	86	11.24	1.1	0.9	0.12	—	—	—
Cook (1974) [7]	England and Wales	86	9.0	3.0	0.25	0.28	0.029	—	—
Fagerhol and Tenfford (1968) [31]	Latin America	94.23	5.77	—	—	—	—	—	—
Robinet-Levy and Riennier (1972) [32]	France	91	7.9	0.6	—	—	—	—	—
Vandeville *et al.* (1974) [29]	South Africa	94	—	—	—	—	—	5.3	0.7
Olusi *et al.* (1981) [15]	Nigeria	66.86	16.29	0.57	2.0	0.28	—	—	—
Present study (1987)	Nigeria	98	—	1	—	—	—	1	—

differences is not clear although ethnic differences in the studies may be a factor. Furthermore, our methodology is different from these reports and the improved sensitivity could also be responsible for the differences encountered.

The PiLM encountered in this study, although new in this environment, is not new in Africa. The existence of this allele in Africa was first noted by Vandeville *et al.* [29] in Zaire and was said to have migrated from India.

In conclusion, we have found three subjects with homozygous-deficient phenotypes as well as 12 heterozygous-deficient subjects. However, the serum A1AT serum levels were significantly low only in the PiZZ subject. Studies are presently under way on replacement therapy with alpha-1-antiproteinase inhibitor in this category of subjects [30]. However, the results from the various centres are still inconclusive as regards the effect of replacement therapy in patients with established pulmonary diseases. If patients with low serum levels are identified early before the establishment of severe disease, it may be possible to help these patients with replacement therapy.

References

1. Schultze HE, Heide K, Haupt H. Alpha-1-antitrypsin from human serum. Klin Wschr 1962;40:427–39.
2. Fagerhol MK, Laurell CB. The Pi system of inherited variants of serum alpha-1-antitrypsin. Prog Med Genet 1970;7:96–111.
3. Cox DW. New variants of antitrypsin comparison of Pi typing techniques. Am J Hum Genet 1981;33:354–65.
4. Arnaud P, Chapuis-Cellier C, Vottes P, Creyssel R. Alpha-1-antitrypsin phenotype in Lyon, France. Hum Genet 1977;39:63–8.
5. Fagerhol MK, Hauge HE. The Pi system phenotype MP discovery of a ninth allele belonging to the system of inherited variants of serum alpha-1-antitrypsin. Vox Sang (Basel) 1968;15:396–400.
6. Fagerhol MK, Braend M. Serum prealbumin polymorphism in man. Science 1965;149:986–92.
7. Cook PJL. Genetic aspects of the Pi system. Postgrad Med J 1974;50:362–4.
8. Cox DW. A new deficiency allele of alpha-1-antitrypsin: Pi Malton. In: Peetersllfed, ed. Protides of the Biological Fluids. Oxford: Pergamon Press, 1976:375–8.
9. Johnson AM. Genetic typing of alpha-1-antitrypsin by immunofixation electrophoresis. Identification of subtypes of PiM. J Lab Clin Med 1976;87:152–63.
10. Lieberman J. Alpha-1-antitrypsin deficiency and chronic pulmonary disease. N Y State J Med 1976;76:181–6.
11. Allen MB, Ward AM, Parks W. Alpha-1-antitrypsin deficiency due to M Malton Z phenotype: case report and family study. Thorax 1986;41:568–70.
12. Eriksson S. Studies in alpha-1-antitrypsin deficiency. Acta Med Scand 1965;432(Suppl):1–85.
13. Feldmann G, Dignon J, Chahinian P. The liver in alpha-1-antitrypsin deficiency. Digestion 1974;10:162–74.
14. Ojutiku OO, Ladipo GOA. Alpha-1-antitrypsin levels and phenotypes in some healthy Nigerians. Nig Med J 1978;8:511–13.
15. Olusi SO, Ladipo GOA, Ojutiku OO. Alpha-1-antitrypsin phenotypes in a Nigerian population. Afr J Med Med Sci 1981;10:113–17.
16. Ayoola EA. Alpha-1-antitrypsin and chronic liver disease in adult Nigerians. E Afr J Med 1982;59:325–38.
17. Aderele WI, Ojo C, Osanyintuyi VO, Oduwole O. Serum alpha-1-antitrypsin levels in asthmatic children. Afr J Med Med Sci 1985;14:161–7.
18. CIBA Symposium. Terminology, definition and classification of chronic pulmonary emphysema and related conditions. Thorax 1959;14:286–99.
19. Cotes JE. Lung functions: assessment and application. In: Medicine, 4th edn. Oxford: Blackwell Scientific Publications, 1979.
20. Laurell EB. Electro-immunoassay. Scand J Clin Lab Invest 1972;29(Suppl 124):21–37.
21. Cox DW. New variants of alpha-1-antitrypsin: comparison of Pi typing techniques. Am J Hum Genet 1981;33:354–65.
22. Kueppers F, Bearn AG. Possible experimental approach to association of hereditary alpha-1-antitrypsin deficiency and pulmonary emphysema. Proc Soc Exp Biol Med 1966;121:1207–9.
23. Welch MH, Beineche ME, Hammarsten JF, Guenter CA. Alpha-1-antitrypsin deficiency in pulmonary disease. The significance of intermediate levels. Ann Intern Med 1969;71:533–42.
24. Eriksson S. Pulmonary emphysema and alpha-1-antitrypsin deficiency. Acta Med Scand 1964;175:197–205.
25. Hunter CC, Pierce JA, La Borde JB. Alpha-1-antitrypsin deficiency family study. J Am Med Assoc 1968;205:23–6.
26. Tarkoff M, Kueppers F, Miller WF. Pulmonary emphysema and alpha-1-antitrypsin deficiency. Am J Med 1968;451:220–8.

27. Lieberman J. Heterozygous and homozygous alpha-1-antitrypsin deficiency in patients with pulmonary emphysema. N Engl J Med 1969;281: 279–84.
28. Higgins ITT. Tobacco smoking, respiratory symptoms and ventilatory capacity: studies in random samples of the population. Br Med J 1959;1:325–36.
29. Vandeville D, Martin JO, Ropartz C. Alpha-1-antitrypsin polymorphism of a Bantu population: description of a new allele PiL. Hum Genet 1974;21:33–8.
30. Gadek JE, Klein HG, Holland PV. Replacement therapy of alpha-1-antitrypsin deficiency; reversal of protease-antiprotease imbalance with the alveolar structures of PiZ subjects. J Clin Invest 1981;68:1158–65.
31. Fagerhol MK, Tenfjord OW. Serum Pi types in some European, American, Asian and African Populations. Acta Pathol Microbiol Scand 1968; 72:601–8.
32. Robinet-Levy M, Reinnier M. Techniques d'identification des groupes Pi premieres statistiques languedociennes. Prev Fr Transfas 1972; 15:61–72.

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