α_1 Antitrypsin deficiency and liver disease in childhood: genetic, immunochemical, histological, and ultrastructural diagnosis

A. MILFORD WARD AND J. C. E. UNDERWOOD

From the Protein Reference Unit, Department of Immunology, Hallamshire Hospital, Sheffield, and the Department of Pathology, The Medical School, Sheffield

SYNOPSIS α_1 Antitrypsin deficiency is a significant factor in the pathogenesis of neonatal cholestasis and progressive juvenile cirrhosis. The diagnosis may be suggested by the liver biopsy appearances and confirmed by immunochemical analysis of the serum. Genetic counselling of affected families is of importance, as medical treatment is ineffective at the present time.

α_1 Antitrypsin is a glycoprotein (MW 54000) which exhibits considerable electrophoretic and genetic polymorphism (Fagerhol and Gedde-Dahl, 1969): the polymorphic system has been designated the Pi system and now comprises 20 phenotypes. Produced by the hepatocyte, α_1 antitrypsin is a broad-spectrum protease inhibitor, the serum level rising in response to tissue injury. Jacobssen (1955) showed that 90% of the tryptic inhibitory capacity of serum could be attributed to this one protein— α_1 antitrypsin. The association of the deficiency state—phenotype Pi ZZ—with emphysema was defined by Laurell and Eriksson (1963) and subsequently confirmed by other workers. Sharp, Bridges, Krivit, and Freier (1969) first described the association of α_1 antitrypsin deficiency with hepatic disease in childhood, and so went some way to explain the earlier impressions that 'neonatal hepatitis' had familial associations (Laplane, Graveleau, Lods, and Noum, 1964). In this paper we present our recent experience of eight cases, in three of which material was available for detailed histological examination. At present this condition would appear inadequately recognized despite the ease with which diagnosis can be made by immunochemical analysis in conjunction with the biopsy appearances. Genetic counselling is of value in affected families.

Clinical Material

The clinical features in all eight cases were essentially

similar, and are illustrated by reference to the three cases in which liver tissue was available for detailed examination. All the children presented initially with the gradual onset of painless obstructive type jaundice at 2-3 weeks of age, the jaundice persisting for a few weeks before spontaneous resolution. There was no associated fever or 'failure to thrive'. Physical examination at the time of the initial presentation showed an enlarged, often firm, liver but no other abnormality.

CASE 1: M.S., MALE, AGED 8 WEEKS

The jaundice was fading when he was first examined although the liver was enlarged (2-3 cm) and firm. In all other respects he was a healthy male infant.

CASE 2: M.H., FEMALE, AGED 3½ YEARS

Admitted as an emergency with a haematemesis from oesophageal varices. Examination revealed established portal hypertension, with enlargement of the liver (3 cm) and spleen (2 cm). She was anicteric at this stage but gave a history of having had a prolonged episode of jaundice with hepatomegaly in the neonatal period. She died from profound haematemesis and liver failure 10 months after the diagnosis was confirmed.

CASE 3: D.B., FEMALE, AGED 12 WEEKS

She presented with jaundice and hepatomegaly at 3 weeks and gradually deteriorated with deepening jaundice and increasing hepatomegaly until death at 12 weeks. This child had three siblings, one of whom has subsequently been shown to have a homozygous α_1 antitrypsin deficiency (Pi ZZ), having had a

Fig 1 Two-dimensional electrophoresis patterns of the Pi MM, Pi MZ, and Pi ZZ phenotypes. The arrows mark the position of peak 4 of the Pi MM phenotype

similar clinical history in the neonatal period, and now, aged 5 years, has established portal hypertension.

Methods

Serum α_1 antitrypsin levels were determined by single radial immunodiffusion and phenotypes characterized by acid starch gel electrophoresis (Fagerhol, 1968) and by discontinuous starch gel electrophoresis followed by electrophoresis into antibody containing agarose gel (Fagerhol and Laurell, 1967).

Surgical liver biopsies in two cases and necropsy tissue in a third were fixed in 10% formol saline and 5 μ m paraffin-embedded sections stained with

haematoxylin and eosin (H & E), periodic acid Schiff (PAS) with and without prior treatment with diastase, picromallory, phosphotungstic acid haematoxylin (PTAH), and Masson's trichrome.

In two cases additional biopsy material was processed for immunofluorescent examination and electron microscopy. Liver tissue for immunofluorescence was snap frozen and 3 μ m cryostat sections were stained with FITC-conjugated rabbit antisera specific for albumin, α_1 antitrypsin, fibrinogen, IgG, IgA, and IgM. For electron microscopy, thin slices of fresh liver tissue were fixed in neutral phosphate-buffered 3% glutaraldehyde. The tissue was embedded in araldite and thin sections were stained with lead citrate.

No.	Sex	Age	α_1 Antitrypsin (g/l)	Pi Phenotype	Comment
1	M	22	1.70	MZ	Well
2	F	20	1.80	MZ	On oral contraceptives
3	M	2 months	0.24	ZZ	Neonatal cholestasis (case 1)
4	M	32	1.10	MZ	Well
5	F	29	1.44	MZ	Well
6	M	7	1.54	MZ	Well
7	F	3½	0.50	ZZ	Progressive cirrhosis (case 2), died
8	M	35	1.50	MZ	Well
9	F	34	1.10	MZ	Well
10	M	12	1.30	MZ	Well
11	F	3 months	—	presumed ZZ	Neonatal cholestasis (case 3), died
12	F	8	2.40	MM	Well
13	F	5	0.45	ZZ	Progressive cirrhosis
14	M	28	0.90	MZ	Well
15	F	28	0.90	MZ	Well
16	M	5	0.38	ZZ	Progressive cirrhosis
17	F	3	0.32	ZZ	Progressive cirrhosis
18	M	22	0.75	MZ	Well
19	F	22	0.60	MZ	Well
20	M	3	0.78	MZ	Well
21	F	15 months	0.48	ZZ	Well
22	F	3 months	0.50	ZZ	Neonatal cholestasis
23	F	29	1.50	MZ	On oral contraceptives
24	M	4	0.45	ZZ	Progressive cirrhosis

Table α_1 Antitrypsin levels and Pi phenotypes in the six families with affected children

Results

IMMUNOCHEMICAL AND GENETIC STUDIES

All the affected children exhibited the homozygous deficiency state as characterized by serum α_1 antitrypsin levels below 0.50 g/l and Pi phenotypes ZZ (fig 1). Normal serum levels of α_1 antitrypsin range from 1.80 to 5.00 g/l for the normal Pi MM phenotype. Heterozygote, Pi MZ levels range from 0.60 to 1.80 g/l. The parents of the affected children were all of Pi MZ phenotype and had α_1 antitrypsin levels in the intermediate range. The Pi phenotypes and serum α_1 antitrypsin levels of the affected children and their families are shown in the table.

All sera were tested for the presence of Australia antigen (HAA) and antibody and were shown to be negative.

LIGHT MICROSCOPY

Sections stained with haematoxylin and eosin (H &

Fig 3 Hepatocytes showing granular fluorescence separated by clear zones (FITC conjugated antiserum to α_1 antitrypsin $\times 160$).

Fig 2 Edge of regeneration nodule. Many hepatocytes enclose numerous granular or globular inclusion (PAS after diastase $\times 290$).

E) did not display any features that distinguish between α_1 antitrypsin deficiency and uncomplicated hepatitis with fibrosis or cirrhosis. Active liver cell necrosis was rarely obvious in the cases examined. The dominant change was lymphoreticular infiltration and expansion of the portal tracts by fibrous proliferation (fig 2). The PAS-stained sections after prior treatment with diastase showed numerous fine granules or globules within the cytoplasm of hepatocytes at the periphery of the surviving lobules or regeneration nodules (fig 2). The concentration of these inclusions decreased towards the centre of the lobules or nodules. No large intra- or extracellular globules were seen in the material examined. Similar inclusions were not seen in a series of biopsies from children with hepatitis or biliary tract obstruction in the absence of known α_1 antitrypsin deficiency. Sometimes, however, the presence of diastase-resistant, PAS-positive material in Kupffer cells caused superficial confusion.

Fig 4 *The globular inclusions consist of amorphous material of medium electron density bordered by a crenated membrane. In places the membrane appears continuous with the endoplasmic reticulum which contains similar material (electron micrograph $\times 32\,000$)*

IMMUNOFLUORESCENT MICROSCOPY

Examination of the liver tissues with FITC conjugated specific antisera showed the presence of large amounts of α_1 antitrypsin within the hepatocytes. In one case α_1 antitrypsin was localized to periportal hepatocytes whilst in the other the localization was more generalized and involved most of the hepatocytes including those in the centrilobular zones. The specific fluorescence was seen as both fine and coarse granulations, separated by clear, unstained areas probably corresponding to the nuclei and mitochondria (fig 3). No α_1 antitrypsin was detected in an extracellular situation or in the portal fibrous tissue. No specific fluorescence was detected in the hepatocytes with any of the other antisera used. In

case 2 there were numerous IgM-containing plasma cells in the portal fibrous tracts. α_1 Antitrypsin was not detected in a control series of liver tissue from patients without antitrypsin deficiency.

ELECTRON MICROSCOPY

Two cases were examined by electron microscopy. The findings were essentially similar. The cytoplasm of the periportal hepatocytes contained numerous globules up to $3\,\mu\text{m}$ diameter (fig 4). Material within the globules was of uniform medium electron density, with an amorphous texture. Each globule was limited by a rather crenated single membrane which appeared to be in continuity with the endoplasmic reticulum.

Occasional lipid globules and focal cytoplasmic degeneration were also seen. Mitochondria, lysosomes, and peroxisomes (microbodies) showed no consistent abnormality. No globular material was found in sinus lining cells or duct epithelium.

Discussion

α_1 Antitrypsin is a broad-spectrum protease inhibitor, inhibiting not only trypsin but also chymotrypsin, elastase, hyaluronidase, skin collagenase, plasmin, and thrombin. It also shows inhibitory action against proteolytic enzymes derived from leucocytes and microorganisms. Biologically, the protein may be considered to have a protective role in neutralizing enzymes released from dying cells during inflammation and tissue injury. In the normal individual the serum level rises during the acute phase of inflammation and infection, as well as under the influence of hormonal stimulation as in pregnancy and during oral contraceptive medication (Sharp *et al*, 1969). Twenty distinct phenotypes, the Pi system, have so far been described (Fagerhol, 1971). The distribution of Pi phenotypes in the random population suggests that inheritance is governed by 11 codominant alleles probably acting at a single autosomal locus (Fagerhol, 1967). Although three Pi phenotypes have been described in association with chronic obstructive airway disease in young adults—Pi ZZ, SZ, and SS (Fagerhol and Hauge, 1969)—only Pi ZZ has so far been incriminated in neonatal hepatitis and progressive juvenile cirrhosis (Sharp, 1971; Aagaens, Matlary, Eljjo, Munthe, and Fagerhol, 1972; Porter, Mowat, Cook, Haynes, Shilkin, and Williams, 1972; *British Medical Journal*, 1973). Serum α_1 antitrypsin levels are normal in mucoviscidosis (Koch, Schwick, and Storiko, 1965); normal or raised in biliary atresia, tyrosinaemia, and familial cystic disease of the liver; and raised in acute hepatitis (Sharp *et al*, 1969). In adults α_1 antitrypsin levels are normal in alcoholic cirrhosis and chronic aggressive hepatitis, although low levels may reflect progressive hepatocellular failure and decompensated cirrhosis.

The distinctive globules in liver cells have been reported in the homozygous α_1 antitrypsin deficiency state associated either with basal emphysema in young adults or with progressive liver disease in children (Sharp, 1971; Lieberman, Mittman, and Gordon, 1972; DeLellis, Balogh, Merk, and Chirife, 1972). Most authors are agreed that the globules tend to accumulate predominantly in the periportal hepatocytes, and, with the exception of Aagaens *et al* (1972), are PAS positive. The reported histological appearances range from overt florid hepatitis, with or without giant cell formation, to an

established cirrhosis. Varying degrees of liver cell necrosis and cholestasis are present (Aagaens *et al*, 1972; Porter *et al*, 1972). Some authors have considered that the material enclosed within the globules is microfibrillar rather than amorphous (DeLellis *et al*, 1972) but the relationship of the globules to the endoplasmic reticulum is generally agreed. It is important to recognize that globular hyaline inclusions have been described in hepatocytes in a variety of experimental and clinical situations, and similar PAS-positive granular staining will be produced by the accumulation within the hepatocyte of lipofuscin, lipochrome pigment, and copper. Whilst α_1 antitrypsin deficiency may be suggested by the presence of diastase-stable, PAS-positive globules in the periportal hepatocytes, the diagnosis must be confirmed by the appropriate immunochemical investigations.

It is uncertain why some patients with the homozygous deficiency state should get hepatic disease whilst others get pulmonary disease, and a few escape either (see table, no. 21). The average gene frequency for Pi Z in northern Europe is 0.02 (Fagerhol, 1971); the expected incidence of the homozygous Pi ZZ state is therefore 0.4 per 1000. From published reports and our own observations it would appear that 25% of Pi ZZ individuals develop hepatic disease, 55% pulmonary disease, and 15% remain symptom free. It would seem likely that the site of initial tissue injury may predict the target organ, although Aagaens *et al* (1972) suggest that the presence of excess α_1 antitrypsin within the hepatocyte is itself hepatotoxic. This would seem unlikely in view of the report by Lieberman *et al* (1972) of globular inclusions within hepatocytes in patients with emphysema, but without overt liver disease, and of the percentage of individuals who remain symptom free. In the case described by DeLellis *et al* (1972), liver disease became apparent only after exposure to a number of potentially hepatotoxic agents. The non-specific nature of the trigger factor is emphasized by the high incidence of HAA-positive cases reported by Porter *et al* (1972), whilst this series and that of Aagaens *et al* (1972) were uniformly negative for HAA.

There remains the apparent disparity between excessive amounts of α_1 antitrypsin within the hepatocyte and the abnormally low serum level. Sharp (1971) in his review suggested two possible mechanisms, either defective synthesis or defective intracellular transport. The ultrastructural evidence suggests that α_1 antitrypsin accumulates at its site of synthesis within the endoplasmic reticulum. Electrophoretic evidence suggests that amino acid or carbohydrate substitutions occur within the molecule to bring about the marked heterogeneity of

electrophoretic mobility of the different phenotypes. The summation of these two abnormalities would suggest that there exists on the Pi ZZ homozygote a fundamental abnormality of α_1 antitrypsin synthesis, and that this induces, as a secondary phenomenon, an abnormality of intracellular transport.

References

- Aagaens, Ø., Matlary, A., Elgjo, K., Munthe, E., and Fagerhol, K. (1972). Neonatal cholestasis in α_1 antitrypsin deficient children. *Acta Paediat. scand.*, **61**, 632-642.
- British Medical Journal (1973). Alpha₁ antitrypsin deficiency and liver disease in childhood. (Leading article.) *Brit. med. J.*, **1**, 758.
- DeLellis, R. A., Balogh, K., Merk, F. B., and Chirife, A. M. (1972). Distinctive hepatic cell globules in adult alpha-1 antitrypsin deficiency. *Arch. Path.*, **94**, 308-316.
- Fagerhol, M. K. (1967). Serum Pi types in Norwegians. *Acta path. microbiol. scand.*, **70**, 421-428.
- Fagerhol, M. K. (1968). The Pi-system: genetic variants of serum α_1 antitrypsin. *Ser. haemat.*, **1** (1), 153-161.
- Fagerhol, M. K. (1971). Le système des Pi, sa génétique et ses rapports avec les maladies. *Poumon et Coeur*, **27**, 41-51.
- Fagerhol, M. K., and Gedde-Dahl, T., Jr. (1969). Genetics of the Pi serum types. Family studies of the inherited variants of serum α_1 antitrypsin. *Hum. Hered.*, **19**, 354-359.
- Fagerhol, M. K., and Hauge, H. E. (1969). Serum Pi types in patients with pulmonary diseases. *Acta allergol.*, **24**, 107-114.
- Fagerhol, M. K., and Laurell, C. B. (1967). The polymorphism of 'prealbumins' and α_1 antitrypsin in human sera. *Clin. chim. Acta*, **16**, 199-203.
- Jacobssen, K. (1955). Studies on the trypsin and plasmin inhibitors in human blood serum. *Scand. J. clin. Lab. Invest.*, Suppl. **14**, 55-102.
- Koch, F., Schwick, H. G., and Storiko, K. (1965). α_1 antitrypsin Bestimmungen im Serum bei Patienten mit Mucoviscidose. *Klin. Wschr.*, **43**, 1120.
- Laplane, R., Graveleau, D., Lods, C., and Noum, I. (1964). Les hépatites néonatales familiales. *Pediatric*, **19**, 217-230.
- Laurell, C. B., and Eriksson, S. (1963). The electrophoretic alpha₁-globulin pattern of serum in α_1 -antitrypsin deficiency. *Scand. J. clin. Lab. Invest.*, **15**, 132-140.
- Lieberman, J., Mittman, C., and Gordon, H. W. (1972). Alpha₁ antitrypsin in the livers of patients with emphysema. *Science*, **175**, 63-65.
- Porter, C. A., Mowat, A. P., Cook, P. J. L., Haynes, D. W. G., Shilkin, K. B., and Williams, R. (1972). α_1 -antitrypsin deficiency and neonatal hepatitis. *Brit. med. J.*, **3**, 435-439.
- Sharp, H. L. (1971). Alpha₁ antitrypsin deficiency. *Hosp. Prac.*, **6**, 83-96.
- Sharp, H. L., Bridges, R. A., Krivit, W., and Freier, E. F. (1969). Cirrhosis associated with α_1 antitrypsin deficiency: a previously unrecognized inherited disorder. *J. Lab. clin. Med.*, **73**, 934-939.