α_1 -Antitrypsin deficiency and infantile liver disease

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McPhie, J. L., Binnie, S., and Brunt, P. W. (1976). *Archives of Disease in Childhood*, **51**, 584. α_1 -Antitrypsin deficiency and infantile liver disease. Infantile liver disease with deficiency of serum α_1 -antitrypsin is illustrated by a description of the clinical, biochemical, and pathological findings in two affected families. The simplicity of the diagnostic tests is emphasized. Review of 61 biopsies of liver from children and adolescents provided a further 3 cases. It is prudent to exclude this metabolic defect in children with a history of 'neonatal hepatitis'.

A precise cause of infantile hepatitis and cirrhosis is often difficult to define although occasionally a potentially correctable metabolic defect is discovered, as, for example, in galactosaemia. Recently it has become apparent that deficiency of the serum antiprotease, α_1 -antitrypsin, is not uncommonly associated with such a disease process (Cottrall, Cook, and Mowat, 1974).

α_1 -Antitrypsin is a glycoprotein produced by the hepatocyte. Its activity is determined by a series of allelic genes (the protease inhibitor (Pi) system), and severe deficiency is associated with the phenotype PiZZ (Brunt, 1974). Although the condition is relatively easy to diagnose by immunochemical studies and by liver biopsy, the association probably remains unrecognized in many instances. The purpose of this communication is to describe the clinical and pathological features of two typical affected families, to emphasize the relative simplicity and importance of the diagnostic tests, and to indicate the contribution of this disorder to infantile and childhood liver disease by means of retrospective study of liver biopsy findings over a 13-year period.

Materials and methods

α_1 -Antitrypsin was detected within hepatocytes by the indirect peroxidase technique (Nakane and Pierce, 1966) using monospecific rabbit antihuman- α_1 -antitrypsin antiserum (Behringwerke AG, Marburg-Lahn) and peroxidase-labelled goat anti-rabbit IgG. Initial studies employed both frozen and paraffin-embedded sections of liver from Case 1 to demonstrate successfully

intracytoplasmic α_1 -antitrypsin although the intensity of staining of paraffin sections was slightly reduced. Saline control sections and immunoperoxidase staining of sections from normal infant liver were negative. Serum level of α_1 -antitrypsin were estimated by radial immunodiffusion using commercially prepared kits (Behringwerke M-Partigen plates).

Out of 1031 liver biopsies performed within the Aberdeen Hospitals Group during the period 1960-73, 61 were carried out on children and adolescents (Table I). Histological analysis of this stored biopsy material was undertaken by recutting sections which were prepared subsequently for study using routine staining

TABLE I
Histological diagnosis in 61 liver biopsies in children and adolescents 1960-73

Diagnosis	No.
Dubin-Johnson syndrome	1
Congenital hepatic fibrosis	2
Kala-azar	1
Niemann-Pick disease	1
Gaucher's disease	1
Cystic fibrosis	1
Metastatic tumour (neuroblastoma 2, carcinoma 1, leukaemia 5)	8
Hepatocellular carcinoma	1
Hepatitis (herpes simplex 1, viral 3, chronic persistent 2)	6
Cirrhosis (haemochromatosis 2, biliary 5, cryptogenic 2)	9
No abnormality*	21
Fatty change	3
Haemosiderosis	1
Cholestasis	2
α_1 -antitrypsin deficiency†	3
Total	61

*Including 2 patients with Gilbert's disease.

†1 patient with 'neonatal hepatitis', 2 with 'juvenile cirrhosis'.

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methods: H & E, Masson's trichrome, Gordon and Sweet's reticulin, and PAS (with and without diastase pretreatment).

Case reports

Family 1.

Case 1. The proband was a male infant born in 1970 of unrelated parents by forceps delivery at 39 weeks; birthweight 2.3 kg. At age 4 weeks he was first admitted to hospital with a 5-day history of increasing jaundice, pale stools, and dark urine. He was icteric (bilirubin 13.3 mg/100 ml) with hepatomegaly (but no splenomegaly); Hb was 12.2 g/dl; there was no blood group incompatibility. The jaundice faded in about 3 weeks. At age 2½ years he was readmitted with a 3-week history of tiredness and anorexia. There was hepatosplenomegaly, and excess urobilinogen and bilirubin in the urine. The relevant results of investigations are shown in Table II. Liver biopsy showed portal fibrosis with focal erosion of the limiting plates and occasional passive septum formation; frank cirrhosis was not present. Tests for autoantibodies in serum, abnormal copper metabolism, and hepatitis B antigen were negative.

TABLE II

Case 1. Haematological and biochemical data

	Age (months)	
	27	47
Haemoglobin (g/dl)	7	12.9
Reticulocyte count (%)	6.6	5.6
Bilirubin (mg/100 ml)	1.4	1
SGOT (IU)	80	84
Alkaline phosphatase (KAU)	34	62
Total protein (g/100 ml)	5.6	7.6

He remained in poor health until readmission at 3 years 11 months with failure to thrive. His height was 91.5 cm (<3rd centile) and his weight 14 kg (<10th centile). Enlarged liver and spleen were again noted and the results of investigations are shown in Table II. A further liver biopsy showed the presence of a micronodular cirrhosis with many hepatocytes, especially those adjacent to connective tissue septa, containing granular or globular intracytoplasmic inclusions which were PAS-positive despite prior treatment with diastase (Fig. 1). Similar but less frequent inclusions were found on review of the first biopsy. The serum α_1 -antitrypsin level was <50 mg/100 ml and his phenotype was PiZZ.

Case 2. An uncle of Case 1, was born at home in 1958 after an uneventful pregnancy; birthweight 3.2 kg. Transient jaundice appeared at 48 hours, becoming progressive at 3 weeks together with troublesome vomiting. The serum bilirubin was 4.5 mg/100 ml but no haematological abnormality was found. A tentative diagnosis of biliary atresia was made and laparotomy was

FIG. 1.—Case 1. Numerous PAS-positive globular inclusions present within cytoplasm of hepatocytes. (PAS after diastase. $\times 150$.)

undertaken at 6 months of age. The gallbladder, cystic duct, and common bile duct appeared normal, although the surgeon considered the right and left hepatic ducts to be possibly atretic and injection of saline led to minimal distension of the ducts. The liver was enlarged but no biopsy was done. Jaundice faded slowly until at 9 months the infant seemed well.

At 4 years he was readmitted with abdominal distension and vomiting, and at laparotomy adhesions were divided and a gastroenterostomy fashioned. Hb 10.5 g/dl, bilirubin 1 mg/100 ml, alkaline phosphatase 38 KAU, and SGOT 180 IU. A wedge biopsy of liver showed a quiescent micronodular cirrhosis; PAS-positive inclusions were present in some hepatocytes. The spleen was not enlarged and oesophageal varices were not demonstrated radiologically.

During the next 2 years progressive splenomegaly was noted and at age 6 years he began to have recurring variceal haematemesis which culminated in a third laparotomy when splenorenal anastomosis and splenectomy were performed. However, he continued to suffer from haematemesis until age 10½ years, when, after uncontrolled major bleeding, further operation proved unsuccessful and he died in renal and hepatic failure. Post-mortem examination was refused.

Family 2.

Case 3. The proband was a male infant born in 1964, birthweight 3.5 kg, after an uncomplicated pregnancy. Progressive jaundice did not appear until 5 weeks of age when hepatomegaly (but no splenomegaly) was noted. The results of relevant investigations at this time are shown in Table III. With conservative measures the jaundice faded gradually over the ensuing weeks. In his third year progressive hepatosplenomegaly was noted. Despite abnormal liver function (Table III) he developed well, remaining relatively asymptomatic save for occasional abdominal pain. When he was 10 years the persistent and increasing hepatomegaly was further investigated (Table III).

TABLE III
Case 3. Haematological and biochemical data

	Age		
	5 weeks	3 years	10 years
Haemoglobin (g/dl)	11.5	10.6	12.2
Reticulocyte count (%)	6	—	4
Bilirubin (mg/100 ml)	3.8	0.4	0.9
SGOT (IU)	125	58	28
Alkaline phosphatase (KAU)	20	23	12

Tests for serum autoantibodies, abnormal copper excretion, and hepatitis-B antigen were negative. Liver biopsy showed a macronodular cirrhosis in which large areas of disorganized hepatic parenchyma were occasionally delineated by a thin rim of connective tissue. PAS-positive inclusions were present in hepatocytes, and these morphological features were consistent with the simultaneous findings of a low serum α_1 -antitrypsin (<50 mg/100 ml) and phenotype PiZZ.

Case 4. The brother of Case 3, was born in 1966; birthweight 3.4 kg. Transient jaundice was present at 48 hours and increased until admission at 6 weeks with vomiting and hepatomegaly (Table IV). Viral studies and WR were negative. Jaundice was maximal at 8 weeks and then gradually faded until the patient became anicteric by 14 weeks. Progress was satisfactory until 27 months when abdominal distension due to ascites and hepatosplenomegaly necessitated admission. Investigation (Table IV) also included a splenic portogram, which showed a patent portal vein with a possible

TABLE IV
Case 4. Haematological and biochemical data

	Age	
	6 weeks	27 months
Haemoglobin (g/dl)	9.6	10
Reticulocyte count (%)	5.6	—
Bilirubin (mg/100 ml)	8.1	0.9
SGOT (IU)	201	102
Alkaline phosphatase (KAU)	28	62

cirrhotic pattern. Splenic pressure was not measured. Ascites was controlled with frusemide and spironolactone.

He was admitted 9 months later with increasing drowsiness and ascites. He was jaundiced, with easily palpable hepatosplenomegaly. Although his conscious level gradually improved with treatment residual ascites, oedema, and jaundice remained and he died with hepatic failure and terminal haematemesis at 37 months. At necropsy, a litre of clear fluid was found in the peritoneal cavity, there were oesophageal and gastric varices, and the stomach was distended by altered blood clot. The

liver (300 g) was grossly cirrhotic, and histological examination showed PAS-positive inclusions within some hepatocytes.

Case 5. The sister of Cases 3 and 4, was born in 1971; birthweight 2.8 kg. The jaundice noted at 48 hours persisted over the first week of life and then gradually faded. She was admitted at 4 weeks with progressive jaundice and hepatosplenomegaly. There was no blood incompatibility, Hb was 10.4 g/dl, reticulocytes 2.8%, serum bilirubin 8.2 mg/100 ml, SGOT 73 IU, and alkaline phosphatase 21 KAU. Her jaundice reached maximum at 6 weeks and then faded completely over the ensuing 3 weeks. When seen at 18 months she seemed healthy with normal development and no hepatosplenomegaly.

Discussion

The characteristics and possible pathogenesis of α_1 -antitrypsin deficiency liver disease have been recently reviewed (Feldman, Bignon, and Chahinian, 1974; Brunt, 1974). Our families illustrate the typical features. The disease commonly manifests itself in the first 3 to 4 weeks of life as a 'neonatal hepatitis' which may be mild, and even pass unrecognized, or more severe, occasionally leading to early death. Cholestasis is common and sometimes severe (Aagaes *et al.*, 1974) so that biliary atresia may be suspected. This is well illustrated in case 2, where, even at laparotomy, the surgeon suspected extrahepatic biliary atresia because of the 'poor distensibility' of the right and left hepatic ducts. Cholestasis, when pronounced, may lead to hypoprothrombinaemia and, in consequence, bleeding. Aagaes and his colleagues (1974) have drawn attention to the commonly low birthweight which they suggest indicates pathogenesis *in utero*, although our cases do not illustrate this feature.

The usual pattern thereafter is gradual subsidence of the jaundice in anything from 3 weeks to several months (Cottrill *et al.*, 1974). But clearly cirrhosis develops in most if not all patients (Sharp and Frier, 1972; Aagaes *et al.*, 1974). Then, as with our patients, the child presents later with a complication such as ascites or haematemesis from varices. The outcome is usually fatal owing to bleeding and its complications (as in Case 2) or hepatic coma (as in Case 4).

In none of our patients was liver biopsy performed during the period of presumed 'neonatal hepatitis'. The histological features described by other authors are variable and non-specific with focal necrosis, bile duct proliferation, and an inflammatory infiltrate including plasma cells and eosinophils. Fibrosis is common but in contrast to many other forms of neonatal hepatitis giant cells are rare

FIG. 2.—Case 1. Immunoperoxidase staining of α_1 -antitrypsin in hepatocytes. (a) Control section treated with saline. (b) Adjacent section treated with antihuman- α_1 -antitrypsin, before application of conjugate; note granular cytoplasmic staining, especially in cells adjacent to fibrous tissue. ($\times 250$.)

(Sharp and Frier, 1972). Cirrhosis tends to be of the macronodular type, as in 2 of our patients.

The salient histological feature in the liver of a patient with α_1 -antitrypsin deficiency is the pre-

sence within some hepatocytes of granular or globular intracytoplasmic inclusions which, characteristically, are PAS-positive despite prior treatment with diastase (Fig. 1). Although virtually

unique to this condition, the presence of such inclusion bodies is not absolutely diagnostic and the demonstration of α_1 -antitrypsin retention must be confirmed by either immunofluorescent or immunoperoxidase (Fig. 2) techniques. Antitrypsin deficiency is confirmed by finding a low serum level and by Pi typing.

The family pattern of the disease is in keeping with its known genetic nature. Superficially it resembles a simple autosomal recessive pattern, which it was originally thought to be (Kueppers, Briscoe, and Bearn, 1965). Since each of a number of different allelic genes is now known to determine a specific amount of circulating α_1 -antitrypsin (except in the case of the very rare PiO gene) the inheritance is more accurately described as codominant. The level in the proband (just below 50 mg/100 ml) is consistent with his known phenotype PiZZ. Those of the parents (obligate heterozygotes for the PiZ gene) are in the intermediate range (170–190 mg/100 ml) and are consistent with the parental phenotype of PiMZ, PiM being the designation of the normal α_1 -antitrypsin gene. Phenotyping is beyond the scope of a routine laboratory but α_1 -antitrypsin levels are easily measured on immunodiffusion plates available cheaply and commercially (Behringwerke Partigen plates, Hoechst Pharmaceuticals).

The extent of the problem is difficult to quantify. In a prospective study of 52 patients with a history of 'neonatal hepatitis' Cottrall *et al.* (1974) discovered 7 patients who were α_1 -antitrypsin deficient (phenotype PiZZ). The present retrospective study of 61 biopsies of liver from adolescents and children has found 3 patients (5%) (Table I) who have histological and immunoperoxidase stigmata of α_1 -antitrypsin liver disease. Two of them were sibs who died at the age of 9 years and 14 years

respectively with haematemesis from oesophageal varices associated with 'juvenile cirrhosis'. The third patient died at the age of 3 weeks during the period of 'neonatal hepatitis'.

The association of serum α_1 -antitrypsin deficiency and cirrhosis is now well established and the diagnosis is readily made from the serum levels and the identification of the characteristic inclusions in hepatocytes. Commercially available antisera to human α_1 -antitrypsin are available for confirmatory immunological staining techniques.

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