Dubin-Johnson syndrome with some unusual features in a Chinese family

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SUMMARY Three cases of chronic nonhaemolytic jaundice with conjugated bilirubin in the serum are described in a Chinese family. Bromsulphthalein excretion tests gave results typical of the Dubin-Johnson syndrome. Liver histology in the proband showed cytoplasmic pigment of the lipofuscin-melanin variety, and intravenous cholecystography failed to show visualisation of the gallbladder. Unusual findings included onset during the neonatal period in the proband and the presence of some iron pigment in the hepatic cells with a little canalicular cholestasis. It is suggested that the infant may have had a concomitant nonspecific hepatitis. These cases are regarded as belonging to a disease group in which the Dubin-Johnson syndrome is at one end of a spectrum. The mode of inheritance is discussed.

The hereditary defects in bilirubin metabolism are rare and incompletely understood causes of jaundice. They can be divided into two groups according to whether the raised serum bilirubin level occurs only in the unconjugated form as in Gilbert's disease (Berk *et al.*, 1970) and Crigler-Najjar disease (Crigler and Najjar, 1952), or in both the conjugated and unconjugated forms. The best known of the latter group was first described by Dubin and Johnson (1954) and independently by Sprinz and Nelson (1954). It is characterised by the accumulation of an unidentified pigment in the liver cells which is probably composed of melanin precursors which polymerise and are stored in lysosomes, although it is often called lipofuscin by pathologists (Arias, 1971). A closely similar clinical and biochemical disorder but without abnormal pigment in the liver cells was first described by Rotor *et al.*, 1948. In this paper we describe three cases of chronic nonhaemolytic jaundice with conjugated bilirubin in the serum in a Chinese family.

The proband

A Chinese male infant, the 4th child of healthy unrelated parents, was born spontaneously in the

United Christian Hospital, Hong Kong on 22 March 1978. The birthweight was 3.58 kg. There was no history of maternal illness, drug ingestion, or x-irradiation. Jaundice was noted on day 2 when the total serum bilirubin (SB) level was 225.7 $\mu\text{mol/l}$ (13.2 mg/100 ml). This rose to 299.3 $\mu\text{mol/l}$ (17.5 mg/100 ml) on day 3 when phenobarbitone and phototherapy were started. Hb was 13 g/dl. G-6-PD deficiency was excluded. The mother's blood group was A Rh +ve, the infant's AB Rh +ve. The infant continued to feed well on a proprietary dried milk formula and on discharge from hospital on 6 April the total SB had fallen to 162.5 $\mu\text{mol/l}$ (9.5 mg/100ml).

He was readmitted to the United Christian Hospital on 24 April because of persisting jaundice with a total SB of 162.5 $\mu\text{mol/l}$ (9.5 mg/100 ml) of which 116.3 $\mu\text{mol/l}$ (6.8 mg/100 ml) was in the conjugated form. Weight was 4.5 kg. The liver was palpable 2 cm below the right costal margin and spleen was just palpable. The urine was positive for bile intermittently but there was no excess urobilinogen on qualitative testing; there were no reducing substances. Amino-acid chromatography was normal. Hb was 10.6 g/dl; white cell count $13.0 \times 10^9/\text{l}$; neutrophils 15%; lymphocytes 75%; monocytes 8%; eosinophils 2%; reticulocytes 2.6%; platelets $490 \times 10^9/\text{l}$. Direct Coombs's test was negative. Red cell fragility normal. ESR (Westergren) 6 mm/1st hour. Blood films were negative for Hb H granules. The stools were normal in colour and consistency.

The patient was transferred to Queen Mary Hospital on 7 June. The results of serial liver function

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tests between 14 June and 10 July are shown in Table 1. Other investigations included: HBsAg negative; serum α -fetoprotein not increased; α -l-antitrypsin 3.6 mmol/min per litre (normal 2.6–3.5); serum thyroxine 108 nmol/l (8.4 μ g/100 ml); IgM 1.22 g/l; IgG 6.15 g/l; IgA 1.50 g/l. Prothrombin time on 14 June and 19 June 11.8 seconds (control 12.0) and 10.5 seconds (control 11.3); partial thromboplastin time 50.0 seconds (control 32.2) and 46.0 seconds (control 31.1). Antibody titres on two occasions two weeks apart showed no evidence of infection with toxoplasma, cytomegalovirus, rubella virus, or herpes simplex. The bromsulphthalein (BSP) test (5 mg/kg) showed 22.9% retention at 30 min, 19% at 60 min, and 30.9% at 120 min.

A Menghini needle liver biopsy on 20 June showed a specimen that was dark green in colour. Histological preparations showed that the liver had a normal lobular pattern. Most of the hepatic cells contained a brown, granular, iron-negative, non-acid fast, PAS-positive (after diastase), golden-brown fluorescent cytoplasmic pigment (Figure).

Figure Granular pigment in liver cells. Note swollen cell just above centre of picture and 2 foci of haematopoiesis at the upper right hand corner. Binucleation and mild nuclear pleomorphism are also demonstrated. PAS after diastase with orange filter $\times 384$.

Table 1 Serial liver function tests on the proband

	14 June	19 June	27 June	10 July
Total bilirubin				
*C—up to 26 μ mol/l	119.7	121.4	80.4	58.1
Conjugated bilirubin				
C—up to 7 μ mol/l	106	111.2	70.1	47.2
Alkaline phosphate				
C—35–115 μ mol/min per litre	1067	738	350	308
Serum AST				
C—13–20 μ mol/min per litre	94	42	56	38
Serum ALT				
C—6–29 μ mol/min per litre	37	34	61	30
Albumin				
C—31–51 g/l	46	43	43	42
Globulin				
C—23–40 g/l	19	20	23	23

*C—Normal values in Chinese in Queen Mary Hospital laboratory. Conversion: SI to traditional units—bilirubin: 1 μ mol/l = 0.0585 mg/100 ml.

Many cells also contain a little iron pigment. There are some cells with canalicular cholestasis, slight ballooning, binucleation, and variation in nuclear size. Other findings are minor and include negligible extramedullary haematopoiesis, a few mononuclear leucocytes in some portal areas, as well as iron pigment, and pigment with above-described characteristic in Kupffer cells.

An intravenous cholecystogram (0.6 ml Biligrafin/kg) when the total SB was 58 μ mol/l (3.4 mg/100 ml) failed to show any visualisation of the gall-bladder up to 4 hours.

During his stay in hospital the infant fed well and gained weight normally (6.0 kg aged 15 weeks). The icterus gradually lessened (Table 1) and the mild hepatosplenomegaly receded. The urine showed bile pigment initially but Erlich's aldehyde test repeatedly failed to show excess urobilinogen. The stools remained normal in colour and consistency throughout.

Table 2 Liver function tests on the proband and other members of the family

	Proband (12 weeks)	Father (43 years)	Mother (33 years)	Sister (11 years)	Brother 1 (10 years)	Brother 2 (6 years)
Total bilirubin (μ mol/l)	119.7	20.8	17.1	17.1	59.9	58.1
Conjugated bilirubin (μ mol/l)	106	8.6	0	0	35.9	27.4
Alkaline phosphatase (μ mol/min per litre)	1067	50	70	405	336	329
Serum AST (μ mol/min per litre)	94	18	24	18	21	26
Serum ALT (μ mol/min per litre)	37	9	25	9	9	13
BSP % retention						
30 min	22.9	2.7	0	15.1	5.6	10
120 min	30.9	0	0	0	10	17.5

Conversion, see footnote to Table 1.

Family study

Both father (43 years) and mother (33 years) are ethnic Chinese and in good health; neither has a history of jaundice. There is no history of jaundice in the father's brother or 4 sisters, nor in the mother's 4 brothers. Their 11-year-old daughter has no history of jaundice and shows no abnormality on clinical examination. Their 10-year-old son had neonatal jaundice which persisted for longer than one month. Since then he has been subject to attacks of abdominal pain, nausea, and anorexia. Clinical examination showed a well developed boy with scleral icterus as the only abnormality. The younger 6-year-old brother had no history of jaundice but clinical examination detected undoubted scleral icterus. The results of biochemical tests on members of the family are shown in Table 2. Liver biopsy on the children was not regarded as justifiable. Unfortunately the grandparents were not available in Hong Kong.

Discussion

The presence of 'lipofuscin-melanin' pigment in the liver cells of the proband and the abnormal BSP tests in the proband and his two older brothers, who demonstrated scleral icterus, are compatible with a diagnosis of the Dubin-Johnson syndrome (DJS). Rotor's syndrome (RS) can be excluded on the liver biopsy finding of pigment deposition. There are, however, several features in the proband which are not typical of DJS. Most patients have first presented in adolescence or adult life, often with abdominal discomfort, nausea, and tiredness (Dubin, 1958; Shani *et al.*, 1970). Onset in the first 48 hours of life is exceptional, although this was the case in a Japanese neonate (Kondo *et al.*, 1975). The initial high SB levels are also atypical but they might be explained on the assumption that an early, presumably unconjugated, hyperbilirubinaemia may have been aggravated by DJS. More significant, perhaps, was the finding in the liver biopsy that while most of the cytoplasmic pigment was 'lipofuscin-melanin', some was apparently iron, whereas in typical DJS all of the pigment is 'lipofuscin-melanin'. The presence of some intrahepatic cholestasis is also not an expected finding in DJS. A possible explanation for these findings might be that this infant had a concomitant nonspecific cryptogenic nongiant cell hepatitis, particularly as there can be considerable variation in the histological appearances in neonatal hepatitis (Talbot and Mowat, 1975) and, at least in patients with α -1-antitrypsin deficiency, subclinical 'biochemical' hepatitis has been demonstrated (Sveger, 1976). The raised alkaline phosphatase and mildly

raised transaminase levels could have a similar explanation although the normal ranges for these values in Chinese newborn infants are not known. On the other hand, some increase in the levels of alkaline phosphatase and transaminases have been reported in other cases of DJS (John and Knudtson, 1956; Dubin, 1958; Shani *et al.*, 1970).

None the less, the results of the BSP tests in the proband and his two male siblings are characteristic of DJS, in particular the secondary rise in BSP concentration at 120 min (Mandema *et al.*, 1960; Arias, 1961; Shani *et al.*, 1970). This pattern of BSP excretion is commonly regarded as diagnostic of the group of familial nonhaemolytic jaundice with conjugated bilirubin in the serum and it is, in fact, the only test which almost invariably gives abnormal results in these disorders (Shani *et al.*, 1970). It has been said not to be found in patients with other liver diseases (Mandema *et al.*, 1960). It would appear that while the uptake of BSP by the liver is normal in the DJS, after attaining saturation point the serum concentration falls more slowly than in normal subjects and after 30-45 minutes again increases so that the value at 120 minutes exceeds that seen at 30-45 minutes. This has been shown to be due to an impairment in the biliary excretion of conjugated BSP and related to a defect in the process by which BSP is transported from the liver cells to the bile. More detailed information can be obtained by sophisticating the BSP test to measure T_m (maximal transport rate) for BSP which is markedly reduced, whereas the relative storage capacity is normal in DJS (Wheeler *et al.*, 1960; Arias, 1961). We were unable to measure these values in the proband because of the volumes of blood which would have been required. The other characteristic but not invariable feature in DJS is absence of visualisation of the gallbladder on cholecystography (Dubin, 1958). This was the finding in the proband in our family, but its significance in the presence of a SB level of 58 $\mu\text{mol/l}$ must be somewhat uncertain. Decreased levels of prothrombin and factor VII have also been reported in DJS (Seligsohn *et al.*, 1970) although in the proband in our family the prothrombin and plasma thromboplastin times were normal.

We believe that the cases in our family should be regarded as examples of DJS, and there is good evidence that the syndrome as originally described by Dubin and Johnson (1954) is but one end of a spectrum of disorders all characterised by a common functional defect in the transport of bilirubin and other substances from the liver cells to the bile. For example, Arias (1961) reported typical cases of DJS with abundant cytoplasmic pigment and of RS with complete absence of intracellular pigment in

two brothers. Butt *et al.* (1966) studied 128 of 242 members of a family in which the classical findings of DJS were present. Pigmentation of hepatic cells was found in only 29 of 39 members who submitted to liver biopsy, but there was no correlation between the amounts of liver pigment and the increases in SB or BSP retention. In 8 members of two Puerto Rican families with jaundice of the DJS variety, Wolf *et al.* (1960) found a striking variation in the amount and distribution of the abnormal pigment. Furthermore, when the accumulation of pigment was slight, jaundice could still be present even without abnormal BSP retention and with normal visualisation of the gallbladder on cholecystography. While delay in the excretion of conjugated BSP with reflux of BSP in the conjugated form into the serum appears to be characteristic of DJS and is related to a defect in the canalicular membrane separating hepatocyte cytoplasm from canalicular bile (Schoenfield *et al.*, 1963), Schiff *et al.* (1959) reported a case of RS in which the BSP retained in the blood was all in the unconjugated form so that in this disorder there would appear also to be a defect in the plasma membrane separating hepatocyte cytoplasm from sinusoidal blood (Edwards, 1975).

The known coexistence of DJS and RS in one family (Arias, 1961) makes it likely that these disorders are variable manifestations of the same mutant gene, and the most generally held view has been that inheritance is in autosomal dominant fashion (Beker and Read, 1958; Mandema *et al.*, 1960; Butt *et al.*, 1966). However, while Butt *et al.* (1966) favoured autosomal dominance they also suggested that the proband in their large family and perhaps one or more of her siblings may have been homozygous for the gene whereas all the other subclinically affected persons were heterozygotes. Pereira Lima *et al.* (1966) concluded from a study of six generations of a family in which 3 members had RS, that an autosomal recessive gene was involved. On the basis of pedigree data obtained on 89 patients of 58 DJS sibships Shani *et al.* (1970) concluded that, at least in Iranian Jews, the mode of inheritance is autosomal recessive. More recently, Shani *et al.* (1973) have extended their study to 114 patients from 63 unrelated kindreds (34 Iranian) with extremely strong support for an autosomal recessive mode of inheritance, and showing a remarkably high rate of consanguinity among the parents in these kindreds. Edwards (1975) reported on the families of 44 American patients who fulfilled strict criteria for DJS. Parental consanguinity was found in at least 11 cases. He also concluded that DJS is inherited as autosomal recessive on the grounds of high frequency of consanguinity, the incidence of the syndrome among siblings of probandi, and the lack

of involvement of more than one generation within each family. Edwards (1975) suggested that reports of cases inherited as autosomal dominants were probably due either to erroneous diagnoses of probandi or their relatives, or to pseudodominant inheritance of the recessive trait where an affected homozygous parent is married to an unaffected heterozygote. In our family the presence of three affected children of apparently normal parents is also compatible with an autosomal recessive inheritance. It is interesting to speculate whether the marginally raised level of conjugated bilirubin in the father's serum ($8.6 \mu\text{mol/l}$) might reflect heterozygosity for the gene.

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