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Case report

Dubin-Johnson-like black liver with normal bilirubin level

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Black liver is a common finding in Dubin-Johnson syndrome (DJS), which is caused by the lack of multidrug resistance-associated protein 2 (MRP2). Impaired excretion of epinephrine metabolites is believed to be a cause of black liver in DJS. Recently, we experienced a patient with black liver whose serum bilirubin level was normal. Coarse brown granules were observed in the hepatocytes, and this finding closely resembled that observed in DJS. However, the granules were negative for Schmorl staining. The *MRP2* gene did not show any mutation. Immunostaining study demonstrated MRP2 protein expression in the liver, and it was localized in the canalicular membranes of hepatocytes. This case illustrates for the first time that DJS is not the only cause of black liver.

Key words: black liver, Dubin-Johnson syndrome, multidrug resistance-associated protein 2, bilirubin

Introduction

Dubin-Johnson syndrome (DJS) is defined as a familial conjugated hyperbilirubinemia.[1,2] This syndrome is characterized by moderate elevation of serum conjugated bilirubin concentration, black liver, coarse brown granules in hepatocytes, negative image on oral cholecystography, rebound elevation of serum sulfobromophthalein (BSP), and by marked increases of urinary coproporphyrin isomer I and leukotriene E_4.[2–4] Multidrug resistance-associated protein 2 (MRP2) is defective in DJS.[3] Several mutations have been identified in the *MRP2* gene from patients with DJS.[5–11] To date, black liver has been reported only in patients with DJS. Here, we report a patient with black liver without DJS, showing, for the first time, that black liver can also be observed in diseases other than DJS.

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Case report

A 35-year-old Japanese man consulted his family physician because of low-grade fever and general fatigue. He was diagnosed with moderate liver injury (alanime aminotransferase [ALT], 238 IU/l; aspartate aminotransferase [AST], 215 IU/l). Three weeks after the onset of his disease, he was admitted to our hospital for further examination. There was neither a personal nor a familial history of liver disease. He had not previously received blood transfusion. He was not being treated with any medicine, and had no history of alcohol drinking. On physical examination, there was no abnormal finding, except of slenderness (body mass index [BMI], 15.04). The conjunctiva and skin were not icteric.

Laboratory data on admission are shown in Table 1. Serum total and conjugated bilirubin concentrations were normal. Serum ALT and AST had already decreased to the normal levels. Viral markers for hepatitis A virus (HAV), hepatitis B virus (HBV), and hepatitis C virus (HCV) were negative. Anti-mitochondrial antibody (AMA) and anti-smooth muscle antibody (ASMA) were negative, but borderline elevation of anti-nuclear antibody (ANA) was observed.

No abnormal observations were found on abdominal ultrasonography (US), abdominal computed tomography (CT), or on magnetic resonance imaging (MRI). Based on these studies, a diagnosis of recovery state from acute hepatitis of unknown cause was made.

On the second hospital day, he underwent a US-guided liver biopsy, which revealed a black tissue specimen. Coarse brown granules were observed in the hepatocytes. Then, abdominal laparoscopy was performed for further evaluation of the liver pigments. On

Table 1. Results of laboratory investigations on admission to our hospital

Peripheral Blood	
WBC	3450/μl
RBC	463×10^4/μl
Hb	16.3 g/dl
PLT	26.3×10^4/μl
Viral markers	
HBs-Ag	(−)
HBs-Ab	(−)
HBc-Ab	(−)
Anti-HCV	(−)
IgM-HA Ab	(−)
IgM-HBc Ab	(−)
Immunology	
ANA	(−)
AMA	(−)
ASMA	(−)
Blood chemistry	
Total protein	6.7 g/dl
Albumin	4.5 g/dl
BUN	6 mg/dl
Creatinine	1.0 mg/dl
Total bilirubin	0.6 mg/dl
Conjugated bilirubin	0.2 mg/dl
AST	39 IU/dl
ALT	25 IU/dl
Gamma-GTP	49 IU/dl
ALP	106 IU/dl
LAP	39 IU/dl
LDH	167 IU/dl
Amylase	104 IU/dl
Serum iron	190 μg/dl
UIBC	191 μg/dl
Total cholesterol	190 mg/dl
Plasma glucose	83 mg/dl
Urinalysis	
Protein	(−)
Sugar	(−)
Urobilinogen	0.1
Ketone	(−)
Occult blood	(−)

WBC, white blood cell count; RBC, red blood cell count; Hb, hemoglobin; HBs, hepatitis B virus surface; Ag, antigen; Ab, antibody; HBc, hepatitis B virus core; HCV, hepatitis C Virus; HA, hepatitis A; ANA, anti-nuclear antibody; AMA, anti-mitochondrial antibody; ASMA, anti-smooth muscle antibody; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; gamma-GTP, gamma-guanosine 5′-triphosphate; ALP, alkaline phosphatase; LAP, leucine aminophosphatase; LDH, lactate dehydrogenase; UIBC, unsaturated iron binding capacity

laparoscopy, the liver showed a sharp edge, smooth surface, and normal size. However, the surface of the liver was black (Fig. 1). Liver histology demonstrated mild inflammatory cell infiltration in the portal area. Coarse brown granules were present in the hepatocytes (Fig. 2a), particularly in the central area. The granules were positive for Fontana-Masson melanin staining[12] (Fig. 2b), but negative for Schmorl's ferric-ferricyanide reduction staining,[13] Berlin blue staining, and Hall's bile pigment staining.[14] These findings suggested that the brown granules contained pigments different from those observed in DJS.

Indocyanine green (ICG) and BSP tests were also performed. The 15-min retention of serum ICG concentration was slightly elevated (12.6%; normal, ≦10%). Rebound elevation of serum BSP concentration was not observed. On oral cholangiography, the gallbladder was well visualized at 12 h after the taking of iopanoic acid. Hepatobiliary scintigraphy, using ^{99}mTc-pyridoxil-5-methyltryptophan, showed no excretion delay. However, the coproporphyrin isomer I fraction in the urine was high, at 79%. Urinary excretion of leukotriene E_4, a major urinary metabolite of leukotriene C_4, was 192.2 pg/mg creatinine (Cr), which was higher than the value determined in a normal subject (97.1 pg/mg Cr) and that in a patient with DJS (175.3 pg/mg Cr).

Direct sequencing of the *MRP2* gene from the patient's genomic DNA was performed, as previously described,[6] and revealed that there was no mutation in the 32 exons or in the promoter region of the *MRP2* gene. Immunohistochemical study, using a monoclonal antibody to MRP2 (MIII-6; ALEXIS JAPAN, Tokyo, Japan) demonstrated normal distribution of MRP2 protein in the liver (Fig. 3).

He was discharged on the twenty-eighth hospital day, and is being followed up without medication at our department.

Discussion

DJS is caused by the lack of MRP2, which is localized in the canalicular membranes or hepatocytes. Impaired excretion of epinephrine metabolites is believed to produce the black liver in DJS.[15] The present patient showed a black liver and brown granules in the hepatocytes, thus resembling findings observed in DJS. Increased urinary excretion of leukotriene E4 and increased fraction of coproporphryn isomer I in the urine are also common findings in DJS. However, in our patient, the brown granules were negative for Schmorl staining. Serum total bilirubin level was normal, and the BSP test showed no rebound elevation of serum BSP. Furthermore, expression of MRP2 protein was detected in the liver, and *MRP2* gene analysis showed no mutation. These findings are not compatible with DJS. These observations suggest that there is a defect in the metabolic pathway of some organic anions of the MRP2 pathway that mediate the transport of leukotriene C4 and coproporphyrins, but without involvement of conjugated bilirubin or BSP.

We were not able to identify the cause of the initial liver dysfunction in the patient, because it was transient and had already disappeared by the time of his admis-

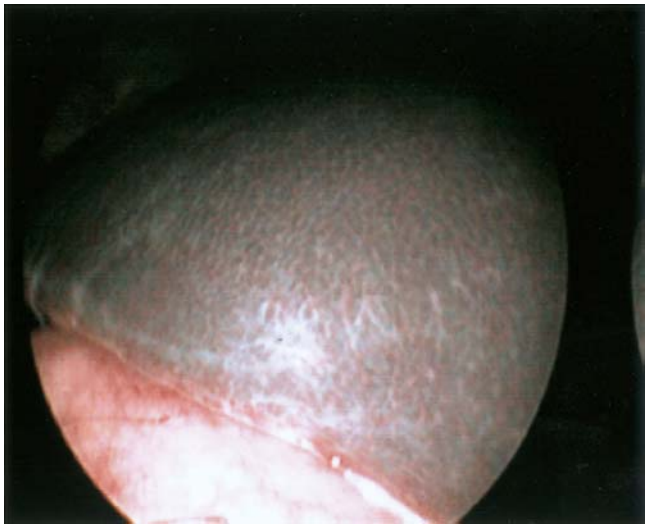


Fig. 1. Laparoscopic image, showing black surface of liver

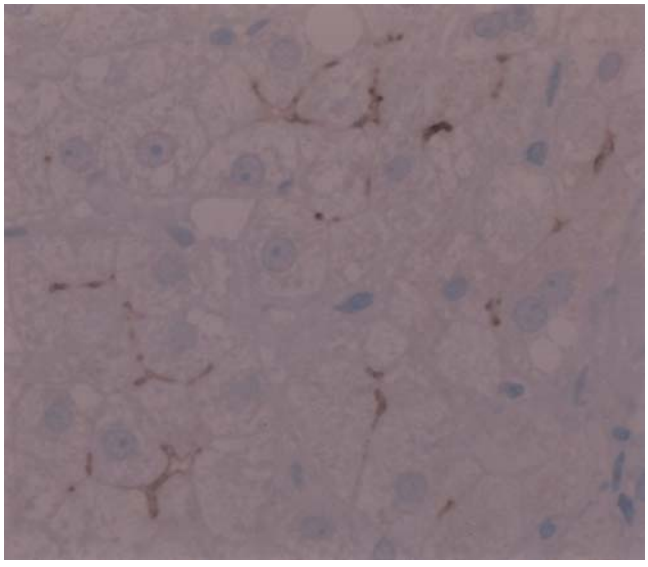
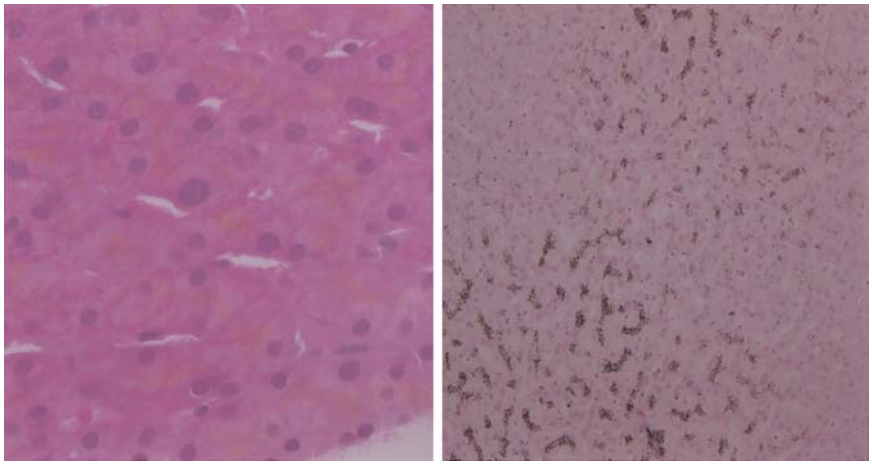


Fig. 3. Immunohistochemical localization of multidrug resistance-associated protein 2 (MRP2) in the liver tissue. Immunohistochemical analysis was performed by immunoperoxidase reaction, with hematoxylin counterstaining. MRP2 was localized in the canalicular membranes of hepatocytes. ×200



a **b**

Fig. 2a,b. Photomicrographs from a needle-biopsied liver specimen. **a** Hematoxylin-eosin staining demonstrated coarse brown granules in the hepatocytes. **b** These granules were positive for Fontana-Masson melanin staining. **a** ×200; **b** ×100

sion. Inflammatory cell infiltration found in the portal area of liver tissue was not specific to determine the etiology of the initial liver injury. The accumulation of brown granules was probably not associated with the initial elevation of serum aminotransferase levels.

This is an interesting case, in that the patient showed a black liver with brown granules different from those observed in DJS. The expression of normal MRP2 protein was also incompatible with DJS. This case illustrates that black liver may be caused by diseases other than DJS.

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