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Original Contributions

Liver pathology in severe multidrug resistant 3 protein deficiency: a series of 10 pediatric cases☆

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ABSTRACT

Multidrug resistance protein 3 (MDR3) is a hepatocyte canalicular membrane protein encoded by the ABCB4/MDR3 gene located on chromosome 7. Several liver diseases are known to be associated with MDR3 deficiency. The basic defect is reduced secretion of biliary phospholipid causing disturbance in the primary bile composition, leading to injury to biliary epithelium inducing cell death and inflammation. Severe MDR3 deficiency typically presents during the first year of life or early childhood, often progressing to chronic liver disease with cirrhosis and portal hypertension, requiring liver transplantation. Negative MDR3 immunostaining is suggestive of MDR3 deficiency. Herein, we report the clinical and histopathologic features of 10 cases (6 male/4 female) in infants and children with severe MDR3 deficiency (age range of 8 months to 7 years) diagnosed with negative MDR3 immunostaining in hepatic canaliculi. Three cases underwent liver transplantation. The cases showed periportal bridging fibrosis to micronodular cirrhosis, ductular proliferation with bile plugs, and lobular canalicular bile stasis with rosetting. All 3 explant livers demonstrated cystically dilated large ducts with crystallization of cholesterol. One case showed well-differentiated hepatocellular carcinoma. We conclude that MDR3 immunostaining on formalin-fixed and paraffin-embedded sections is a useful tool to diagnose severe MDR3 deficiency in pediatric liver cholestatic disease cases where genetic testing is not available.

1. Introduction

Chronic cholestasis is a major cause of liver disease, cirrhosis, and progressive liver failure, ending in liver transplantation or death of the patient. Certain instances of pediatric cholestatic liver disease have been traced to defects in genes that encode proteins expressed at the bile canaliculus required for normal bile flow [1]. There are 3 primary active transport proteins for bile canalicular flow, the adenosine triphosphate–binding cassette transporters ABCB11 encoding the bile salt export pump protein; adenosine triphosphate–binding cassette, subfamily B, member 4 (ABCB4) encoding the multidrug resistant 3 (MDR3) protein; and the P-type adenosine triphosphatase ATP8B1 encoding the familial intrahepatic cholestasis protein 1 [2-4]. Multidrug resistant 3 protein is a phospholipid floppase involved in biliary phosphatidylcholine excretion [4]. Defects in MDR3 protein impair biliary phospholipid secretion, which is required for the formation of mixed micelles in bile resulting in cholestatic liver disease [5-7]. Generally, serum gamma glutamyl transferase levels are generally elevated in MDR3 deficiency patients [1]. Negative MDR3 immunostaining is suggestive of MDR3 deficiency [1]. We report the clinical and histopathologic features of 10 cases of MDR3 deficiency diagnosed with absence of MDR3 immunostaining in hepatic canaliculi. Three cases underwent liver transplantation. We also describe the first case of pediatric hepatocellular carcinoma in severe MDR3 deficiency.

2. Material and methods

From January 1, 2012, to April 30, 2015, all evaluable cases of chronic cholestasis in infants and children diagnosed as MDR3 deficiency were retrieved from the database files of the Department of Histopathology. A total of 10 cases of MDR3 immunostaining negative cases were reported during this period, and all were included in this study. Retrospective clinical and histologic analyses were performed. The material composed of formalin-fixed, paraffin-embedded tissue blocks; tissue sections stained with routine hematoxylin-eosin, periodic acid Schiff, and periodic acid Schiff after diastase; and Masson trichrome, perls, orcien, rhodanine stains, and immunostained slides. Additional sections of 3 to 5 μm were cut and stained with hematoxylin-eosin as required. All slides were reviewed, and the following lesions were assessed: portal and periportal fibrosis (staged according to Scheuer system [8]), portal inflammation (mild, moderate, and severe), ductular proliferation/reaction, cholangitis, rosetting, ductular/canalicular bilirubinostasis, cholesterol cleft in bile ducts, cellular swelling with cytoplasmic rarefaction,

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lobular inflammation (mild, moderate, and severe), and lobular collagenization (focal [<50% of lobular area] or diffuse [≥50% of lobular area]). For immunohistochemical staining, an anti-MDR3 mouse monoclonal antibody (clone P3II-26; Enzo Life Sciences) was used. We used anti-Multidrug Resistance Protein 2 (MRP2) antibody (clone M2 III-6) ab3373 (Abcam) to check canalicular tissue reactivity. Slides were dewaxed in xylene and hydrated through alcohol to water. Endogenous peroxidases were blocked using peroxidase blocking solution at room temperature for 20 minutes. Heat-induced antigen retrieval was done in Decloaking Chamber NXGEN (Biocare) in pH 9 EDTA-Tris buffer for 15 to 20 minutes. Sections were incubated in primary antibody at dilution 1:25 for 1 hour. Normal liver donor biopsies were used as positive controls for MDR3.

3. Results

Ten children (6 male and 4 female) who had undergone liver sampling with a histopathologic analysis (7 needle liver biopsies and 3 explant livers with subsequent orthotopic liver transplantation) and MDR3 immunostaining were included in the study. All trucut biopsies contained more than 7 portal tracts. Tables 1 and 2 summarize the clinical and pathologic findings. Patient's age ranges from 8 months to 7 years. Metabolic, viral, and autoimmune work-up was negative in patients. Patient 1 developed liver dysfunction since age 1 and 1/2 years. She was started on supportive medications. She also had 2 episodes of upper gastrointestinal bleeding. Her trucut liver biopsy was done elsewhere and was reported as micronodular cirrhosis. She was then referred to us for liver transplantation. Her elder brother died at the age of 5 years due to liver disease (details not available). Her total cholesterol was 164 mg/dL. Her α-fetoprotein was 4.19 IU/mL. Cholangiographic studies did not reveal any biliary stricture. She underwent deceased donor split liver transplantation in March 2015. The explant liver weighed 700 g. External and cut surface were greenish micronodular. Multiple large pale-to-greenish macronodules with largest measuring 2×1.5 cm were identified in the right lobe (Fig. 1A). Inspissated bile within dilated intrahepatic bile ducts was also identified grossly (Fig. 1A, arrow). Light microscopy revealed micronodular biliary cirrhosis (Fig. 1B) with expanded portal tracts and septa displaying variable bile ductular reaction, with mild lymphocytic inflammatory cell infiltrate. Associated cystically dilated large biliary channels with focal denudation of epithelium, mild periductular fibrosis, intraductal cholesterol crystals, and bile stasis were also noted in keeping with phospholipid deficiency (Fig. 1C). The lobular parenchyma showed rossetoid arrangement of hepatocytes, focal sinusoidal collagenization, patchy hepatocanalicular bile stasis, ballooning of hepatocytes with rarefied cytoplasm, and few Mallory-Denk bodies. The hepatocytes showed stainable copper in periseptal location. Nodular well-differentiated hepatocellular carcinoma demonstrating thin-trabecular pattern with pseudoglands was identified (Fig. 1D). No microvascular tumor invasion was noted. Dysplastic nodules were also noted. Immunostaining reveals MDR3 predominantly negative to occasional faint canalicular staining. Postoperatively, she developed posterior reversible encephalopathy syndrome with hypertension. As tacrolimus could cause posterior reversible encephalopathy syndrome and hypertension, it was changed to cyclosporine. She was discharged in stable condition and is under regular follow-up for the last 2months.

Patient 2 had strong family history of liver disease. He was apparently well until the age of 2 years when he developed signs and symptoms of liver dysfunction. He is the fifth sibling. The first child died in 2001 at age of 1 month, cause not known. The second child died at 7 years of age in 2010 with an undiagnosed liver disease. The third is 9 years old and healthy. The fourth sibling (patient 3) underwent liver transplant at our center for in 2012 and is described below. He was referred for liver transplant at our center. There was no history of itching or clay colored stools. His total cholesterol was 50 mg/dL. He underwent living donor liver transplant in July 2014. The explant liver weighted 500 g. External and cut surface was greenish micronodular. Inspissated bile within dilated intrahepatic bile ducts was identified occasionally. The sections showed biliary micronodular cirrhosis with moderate bile ductular proliferation and lymphocytic inflammation and mild interface activity (Fig. 2A). Prominent ductular bile plugs were noted (Fig. 2B). Dilated large intrahepatic biliary channels with cholesterol clefts and bile were noted (Fig. 2C). The lobular parenchyma showed diffuse rosetting with hepatocyte clarification and hepatocanalicular bilirubinostasis. Patchy sinusoidal collagenization and stainable copper deposition were appreciated. Immunostaining was negative for MDR3. He is under regular follow-up for last 10 months and is doing well. Patient 3, born of second-degree consanguineous marriage, was evaluated for the complaints of intermittent itching and lethargy, since infancy. She underwent liver biopsy elsewhere, which showed diffuse bridging fibrosis. She was referred to us and underwent living donor liver transplantation at our hospital. Her total cholesterol was 97 mg/dL. She underwent living donor liver transplant in June 2012. The explant liver weighted 500 g. The liver demonstrated bridging fibrosis with patchy micronodularity and bile ductular proliferation. There was rosetting (Fig. 2D) with periportal cholate stasis. Mild lobular inflammation (Fig. 3A, inset) was also identified. Cholesterol clefts with bile were noted in occasional ducts. Periportal/periseptal copper deposition was noted (Fig. 3B). Multidrug resistant 3 was completely negative (Fig. 3C) with diffuse canalicular staining in normal control liver (Fig. 3D). MRP2 showed diffuse strong canalicular positivity (Fig. 4A). She is under regular follow-up at other center and is doing well for the last 3 years.

Patient 4 is the first female child for consanguineous parents. She was apparently normal until age of 3 months, when she developed hemarthrosis of the left elbow joint, which was treated with vitamin K and fresh frozen plasma. She was followed up in other hospital. Three months later, she developed enlarged liver and abnormal liver indices. She was referred to us for evaluation. Her biopsy revealed cirrhosis with diffuse sinusoidal collagenization (Fig. 4B). She was started on ursodesoxycholic acid and is followed up at other center. Patient 5

Table 1
Clinical findings of the 10 patients.

Patient no.	Age	Sex	Presenting complaints	Total/direct bilirubin	GGT[a]	AST/ALT[a]
				mg/dL	U/L	U/L
1	6 y	F	Gradually progressive jaundice and hepatosplenomegaly	5.3/3.9	178	56/158
2	4 y	M	Fluctuating jaundice, abdominal distension, & lethargy	18.3/15.9	67	314/94
3	7 y	F	Intermittent itching and lethargy since infancy	2.1/1.08	161	146/197
4	8 mo	F	Hemarthrosis at 3 mo. Jaundice at 6 mo	3.1/2.3	184	172/96
5	5 y	M	Fluctuating jaundice from last 1 y	2.3/1.7	255	238/158
6	7 y	M	Pruritus, faltering growth, portal hypertension at 4 y	4.2/3.98	359	200/171
7	5 y	M	Fluctuating jaundice since the age of 5 mo, hepatosplenomegaly	4.8/3.9	328	528/323
8	5 y	M	Jaundice, hepatosplenomegaly	5.1/3.1	607	154/107
9	9 mo	M	Jaundice with intracranial hemorrhage and hepatomegaly	16.2/13.1	600	572/189
10	7 y	F	Jaundice	7.6/4.1	215	157/90

[a] Abbreviations: GGT, gamma glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine transaminase.