

The Clinicopathological Parameters for Making the Differential Diagnosis of Neonatal Cholestasis

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Received : August 14, 2008
Accepted : November 7, 2008

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*This study was supported by a grant from the National
Research and Development Program for Cancer
Control, Ministry of Health and Welfare, Republic of
Korea (0620210).

Background : The diseases that cause neonatal cholestasis display several overlapping clinical feature. Making the differential diagnosis using liver biopsy specimens from infants with neonatal cholestasis is important for delivering the proper treatment. **Methods :** We assessed the clinical manifestations, laboratory data, and histopathologic features of the pretreatment liver biopsy specimens from patients suffering with biliary atresia (n=66), intrahepatic bile duct paucity (n=15), and neonatal hepatitis (n=21). **Results :** The gender distribution was nearly equal for the patients with biliary atresia and intrahepatic bile duct paucity, whereas males predominated for the cases of neonatal hepatitis. Only the gamma-glutamyl transferase level differed significantly amongst the groups. The diagnostic features for making the differential diagnosis of bile duct lesions included marked bile ductular proliferation, severe fibrosis, and bile duct loss. The difference of the average percentage of portal tracts with bile duct loss was statistically significant between the patients with intrahepatic bile duct paucity (73.9%) and those patients with neonatal hepatitis (39.1%) ($p<0.001$). **Conclusions :** Bile ductular proliferation, bile duct loss, and advanced fibrosis are useful for the differential diagnosis of neonatal cholestasis. Moreover, stricter diagnostic criteria for bile duct loss (more than 2/3 of bile ducts) should be applied for the definitive diagnosis of intrahepatic bile duct paucity, because bile duct loss also frequently occurs in infants suffering with neonatal hepatitis.

Key Words : Neonate; Cholestasis; Biliary atresia; Hepatitis; Intrahepatic bile ducts

Neonatal cholestasis is a condition of infants in which jaundice, dark urine, and hypocholic or acholic stools persist for more than 2-3 weeks.¹ There is a broad spectrum of diseases that cause neonatal cholestasis and this includes both obstructive and non-obstructive etiologies. Among the obstructive etiologies are biliary atresia, common bile duct obstruction, choledochal cyst with biliary sludge, and an inspissated bile/mucous plug. Among the non-obstructive etiologies of neonatal cholestasis are bacterial and viral infections, metabolic diseases, intrahepatic bile duct paucity, cirrhosis, total parenteral nutrition, drugs, and idiopathic neonatal hepatitis.²⁻⁵ The extent of physiological cholestasis can greatly vary between patients and this can lead to diagnostic delay during an infant's first 3-4 months of life.⁶

Making the differential diagnosis of biliary atresia from other etiologies is crucial for the proper timing of surgical procedures.^{7,8} Making the correct diagnosis can involve laboratory workups, radiological procedures, and liver biopsy, with the latter being

the single most informative investigational tool.¹ The histologic features of biliary atresia include cholestasis, portal tract expansion that's caused by edematous fibroplasias and periportal ductular reaction, giant cell transformation, and acute and chronic inflammation in the portal/periportal areas. The liver biopsy specimens of patients with neonatal hepatitis are characterized by many of the same features, including cholestasis, giant cell transformation, lobular and portal inflammation and progressive fibrosis, as well as by the ballooning of the hepatocytes and extramedullary hemopoiesis.^{6,9} Since these two conditions share many of the same histological features, such as various degrees of cholestasis, giant cell transformation, and portal inflammation with progressive fibrosis, arriving at the definitive diagnosis is often difficult, even for the experienced pathologists.¹⁰ The aim of this study was to identify any histological clues from liver biopsy specimens or any clinical findings that might be helpful for making the differential diagnosis of neonatal cholestasis.

MATERIALS AND METHODS

We selected the liver biopsies from 112 patients who presented with neonatal cholestasis from March 1996 to May 2007 at the Asan Medical Center, Seoul, Korea. The final clinicopathological diagnoses are summarized in Table 1. The most frequent cause of neonatal cholestasis was biliary atresia (66 patients, 58.9%), followed by intrahepatic bile duct paucity, either syndromic (9 patients, 8.0%) or non-syndromic (12 patients, 10.7%), and neonatal hepatitis (15 patients, 13.4%). The remaining 10 patients were diagnosed with progressive familial intrahepatic cholestasis (PFIC), arthrogryposis, renal dysfunction and cholestasis (ARC), and total parenteral nutrition (TPN)-related cholestasis.

The clinical manifestations, laboratory data, hepatobiliary scans, and the various histopathologic features of the pretreatment liver biopsy specimens were assessed for the 66 patients with biliary atresia, for the 21 patients with intrahepatic bile duct paucity, and for the 15 patients with neonatal hepatitis. Except for six patients, from whom two liver biopsies were obtained, all the patients had one pretreatment liver biopsy specimen.

The formalin-fixed, paraffin-embedded tissue samples were stained with hematoxylin-eosin and Masson trichrome, and they were immunostained with antibody to cytokeratin (CK) 7 (1:200, DAKO; Glostrup, Denmark). All the liver biopsy specimens were assessed for hepatocyte changes, including giant cell transformation and hepatocyte ballooning, and portal tract abnormalities, including the degree of portal inflammation, bile duct

tular proliferation, bile duct loss, and fibrosis. Extramedullary hemopoiesis was also assessed.

RESULTS

Clinical features

The gender distribution was nearly equal for the patients with biliary atresia and intrahepatic bile duct paucity, whereas males predominated over females (2:1) for the patients with neonatal hepatitis. The age at liver biopsy was slightly older for the patients with neonatal hepatitis (73 ± 29 days) than that for the other two groups (62 ± 37 and 66 ± 26 days, respectively) (Table 1).

Amongst the various tests for abnormal liver function, only gamma-glutamyl transpeptidase (GGT) showed significant between-group differences. The GGT concentrations were significantly higher in the patients with biliary atresia (432.2 ± 275.0 IU/L) than that in the patients with neonatal hepatitis (198.0 ± 130.0 IU/L) and intrahepatic bile duct paucity (127.6 ± 103.5 IU/L).

Table 2. Histopathological features of neonatal cholestasis

	Biliary atresia (n=66) (%)	Neonatal hepatitis (n=17) (%)	Paucity of intrahepatic bile ducts (n=24) (%)	p-value
Bile duct loss	27 (39.7) ($7.3 \pm 10.6^*$)	15 (88.2) ($39.1 \pm 23.4^*$)	24 (100.0) ($73.9 \pm 19.7^*$)	<0.001
Bile ductular proliferation				
Absent	0 (0)	15 (88)	20 (83)	NS
Present	66 (100)	2 (12)	4 (17)	<0.001
Portoperiportal activity				
None to minimal	22 (33)	5 (30)	11 (45)	NS
Mild	37 (56)	6 (35)	9 (38)	NS
Moderate to severe	7 (11)	6 (35)	4 (17)	NS
Fibrosis				
Absent	0 (0)	2 (12)	3 (12)	NS
Portal	1 (2)	7 (41)	12 (50)	NS
Periportal	21 (32)	6 (35)	7 (29)	NS
Septal	32 (48)	2 (12)	2 (9)	<0.001
Cirrhosis	12 (18)	0 (0)	0 (0)	<0.001
Giant cell transformation				
Absent to mild	38 (58)	10 (59)	17 (71)	NS
Moderate to severe	28 (42)	7 (41)	7 (29)	NS
Extramedullary hemopoiesis				
Absent	29 (44)	2 (12)	11 (45)	NS
Present	37 (56)	15 (88)	13 (55)	NS

Table 1. Clinical manifestations in neonatal cholestasis

	Biliary atresia (n=66)	Neonatal hepatitis (n=15)	Paucity of intrahepatic bile ducts (n=21)	p-value
Sex (M:F)	30:36	10:5	11:10	NS
Age (days)	62 ± 37	73 ± 29	66 ± 26	NS
AST (IU/L)	272.0 ± 198.5	247.5 ± 193.4	190.7 ± 141.9	0.064
ALT (IU/L)	190.9 ± 157.7	192.3 ± 171.8	136.0 ± 99.9	NS
ALP (IU/L)	999.1 ± 647.6	855.3 ± 536.7	902.1 ± 372.8	NS
GGT (IU/L)	432.2 ± 275.0	198.0 ± 130.0	127.6 ± 103.5	<0.001
Total bilirubin (mg/dL)	9.0 ± 2.8	9.7 ± 3.2	9.0 ± 3.3	NS
Direct bilirubin (mg/dL)	5.4 ± 1.8	5.3 ± 1.8	5.1 ± 2.0	NS
Hepatobiliary scan	61/62*	3/14*	10/19*	

*Number of patients with non-visualization of gallbladder and no bowel activity/number of total patients who underwent hepatobiliary scans. NS, not significant.

*Number of portal tracts without bile ducts \times 100/number of total portal tracts. NS, not significant.

Hepatobiliary scans were performed in 62 patients with biliary atresia, in 14 patients with neonatal hepatitis, and in 19 patients with intrahepatic bile duct paucity. All but one patient with biliary atresia (61 cases) showed non-visualization of the gall bladder and no bowel activity. However, 3 patients with neonatal hepatitis and 10 patients with intrahepatic bile duct paucity also showed no biliary excretion.

Histologic findings

The characteristic histopathologic features of the three conditions are summarized in Table 2. Loss of bile ducts (unobserved bile ducts from the portal tract), which is a diagnostic feature of intrahepatic bile duct paucity, was observed in 15 of 17 patients (88.2%) with neonatal hepatitis and in 27 of 66 patients (39.7%) with biliary atresia (Table 2). The average percentage

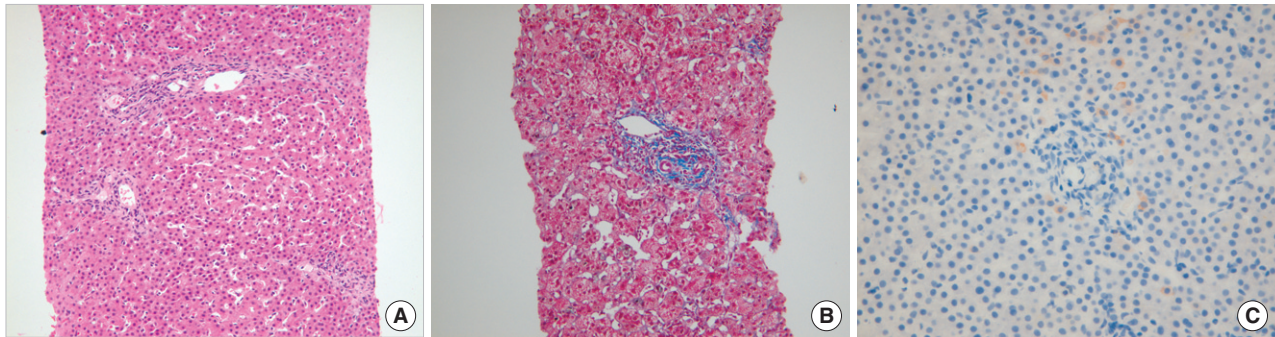


Fig. 1. A representative case of intrahepatic bile duct paucity. (A) Portal tracts are unremarkable except for no recognizable bile duct (Hematoxylin & eosin staining). (B) Portal fibrosis is minimal (Masson trichrome staining). (C) Cytokeratin (CK) 7 immunostaining shows neither bile duct nor ductular reaction.

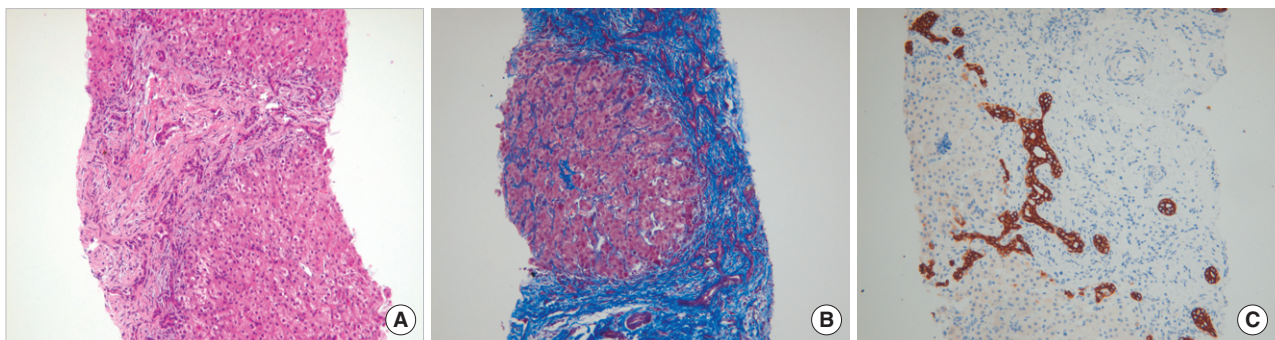


Fig. 2. A representative case of biliary atresia in the cirrhotic stage. (A) Complete septal fibrosis with marginal ductular proliferation is present (Hematoxylin & eosin staining). (B) A cirrhotic nodule with dense fibrosis shows marked bile ductular proliferation (Masson trichrome staining). (C) CK 7 immunostaining delineates proliferating ductules.

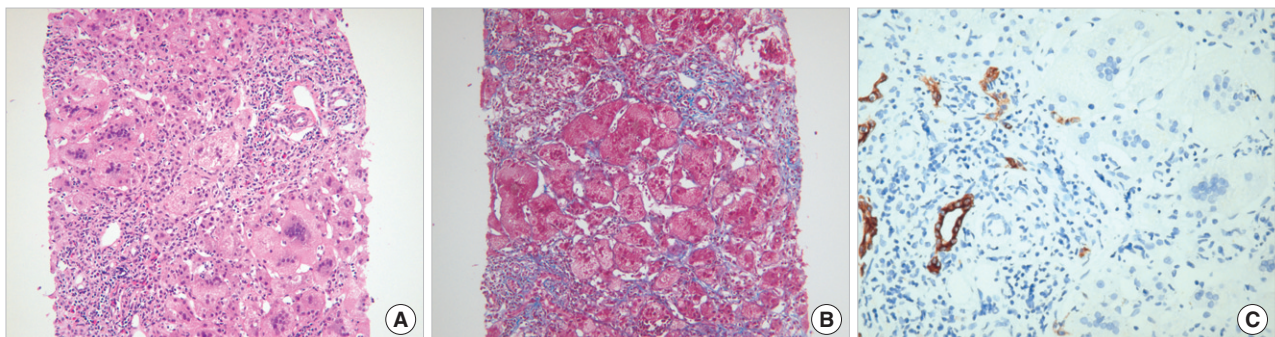


Fig. 3. A representative case of neonatal hepatitis. (A) Giant cell transformation and moderate portal inflammation is present (Hematoxylin & eosin staining). (B) Loose portal fibrosis and pericellular fibrosis is present in Masson trichrome staining. (C) CK 7 immunostaining shows well-formed bile duct and mild ductular reaction.

of portal tracts with bile duct loss was 73.9% in the patients with intrahepatic bile duct paucity (Fig. 1), 39.1% in the patients with neonatal hepatitis, and 7.3% in the patients with biliary atresia ($p < 0.001$). Periportal bile ductular proliferation is a diagnostic feature of biliary atresia (Fig. 2). However, two patients with neonatal hepatitis and four with intrahepatic bile duct paucity also showed ductular proliferation, although this was less prominent than in the patients with biliary atresia. Although various degrees of fibrosis were frequently observed in all three disease conditions, the occurrence of septal fibrosis or cirrhosis was significantly greater in the patients with biliary atresia than that in those patients with neonatal hepatitis and those patients with intrahepatic bile duct paucity ($p < 0.001$). Although portoperiportal inflammation and extramedullary hemopoiesis were observed more frequently in the patients with neonatal hepatitis (Fig. 3), the frequencies of these findings did not significantly differ among the cases of the three disease entities.

DISCUSSION

Biliary atresia, neonatal hepatitis, and intrahepatic bile duct paucity are among the major etiologies of neonatal cholestasis. In the first 3–4 months of life, infants have some degree of physiologic cholestasis because of the inefficient uptake of bile acids and other organic anions by the hepatocytes and the presence of immature hepatocellular pathways for bile acid conjugation and biliary secretion.⁶ Under these circumstances, the immediate priority is to differentiate pathologic cholestasis from the usually benign physiologic forms of this condition.

Making the early diagnosis of the etiology of neonatal cholestasis is crucial because the treatment modalities for the various conditions are quite different and the proper timing of treatment is closely related to the patients' prognosis.¹¹ Although the characteristic pathologic features of the three different etiologies have been well-described, bile duct loss, giant cell transformation of hepatocytes, and portal inflammation occur frequently in the patients with all three conditions.⁶ Although we found that most patients with neonatal hepatitis and more than one-third of those patients with biliary atresia showed a loss of the interlobular bile ducts, the proportion of portal tracts with bile duct loss varied widely, from 7.3% in the patients with biliary atresia to 39.1% in those patients with neonatal hepatitis. Moreover, a study of 16 needle biopsies from normal subjects found that 7% of the examined portal tracts did not contain bile ducts,^{12,13} indicating that the observed frequency of bile duct

loss in the patients with biliary atresia (7.3%) in our study was within the normal range. Intrahepatic bile duct paucity is diagnosed according to the absence of interlobular bile ducts in more than 50% of the portal tracts.⁶ Because of the overlap in the proportions of portal tracts with bile duct loss between the patients with neonatal hepatitis and the patients with intrahepatic bile duct paucity, a higher cut off percentage value should be used for making the differential diagnosis of intrahepatic bile duct paucity, especially of the non-syndromatic type.

Giant cell transformation, ballooning changes in hepatocytes, extramedullary hemopoiesis, and lobular and portal inflammation are among the features of neonatal hepatitis. We found that these findings are also frequently observed in other disease entities. Yet prominent periportal ductular proliferation and septal fibrosis or cirrhosis may be useful for making the differential diagnosis of biliary atresia from the other etiologies of neonatal cholestasis.

Although liver biopsy is the single most informative investigational tool, other methods can be used to differentiate the three etiologies we studied. For example, for the patients <10 weeks old, the peak GGT value was significantly higher in the patients with biliary atresia than that in those patients with neonatal hepatitis (622.5 ± 211.9 IU/L vs 168.8 ± 100.3 IU/L, respectively, $p < 0.001$). A serum GGT concentration >300 IU/L had a diagnostic accuracy of 85% for biliary atresia in the patients <10 weeks old.¹⁴ In agreement with this result, we also observed that GGT was the only reliable laboratory marker for making the diagnosis of biliary atresia.

Hepatobiliary scintigraphy has been used to differentiate biliary atresia from other causes of neonatal cholestasis.^{15–17} However, a nondraining pattern reflects the inability of the liver to excrete the radiotracer. This may be a result of severe hepatocellular dysfunction, defective interlobular bile ducts, or damage to the medium-sized or large bile ducts. Thus, a nondraining pattern may represent several different hepatic diseases, and not just biliary atresia.^{18,19} Our results were similar to the previous studies, that 50% of the patients with interlobular bile duct paucity and 25% of the patients with idiopathic neonatal hepatitis demonstrated no biliary excretion.¹⁸ These results suggest that hepatobiliary scanning requires cautious interpretation and a meticulous histologic examination is necessary to soundly confirm the diagnosis.

In conclusion, performing a histologic examination is crucial for making the diagnosis of neonatal cholestasis. Bile ductular proliferation, bile duct loss, and advanced fibrosis are useful for making the differential diagnosis of neonatal cholestasis. More-

over, stricter diagnostic criteria for bile duct loss (more than 2/3 of the bile ducts) should be applied for making the definitive diagnosis of intrahepatic bile duct paucity, because bile duct loss also frequently occurs in infants with neonatal hepatitis.

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