NEONATAL CHOLESTASIS AND BILIARY ATRESIA: PERSPECTIVE FROM MALAYSIA

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ABSTRACT:

The liver is an important organ of the human body, playing a major role in the metabolism and storage of nutrients, synthesis of protein and other nutrients, as well as detoxifying many metabolic by-products. The response of the foetal and newborn liver to external insult and injury is limited. This is because the ability of the closely interdependent structures of a developing liver of expressing in the face of a variety of insults is limited as well. Thus most infants with insults to the liver present as cholestatic jaundice with variable degree of pale stools, enlarged liver and conjugated hyperbilirubinaemia. Biliary atresia, an idiopathic condition characterized by progressive fibrosing obliteration of both intra- and extrahepatic bile ducts, is the most important cause of neonatal cholestasis worldwide, including Malaysia. It is also the most important indication for childhood liver transplantation the world over. Challenges facing infants with biliary atresia include a delay in the diagnosis and late surgery, leading to a poor outcome. This often results from a failure to recognise the potential serious nature of an infant with prolonged cholestatic jaundice and pale stools among health care professionals. *(JUMMEC 2010; 13(2): 72-79)*

KEYWORDS: *neonatal cholestasis, biliary atresia*

Introduction

The liver is the largest organ of the human body. It weighs approximately 2 to 2.5% of the total body weight of a healthy adult. The liver plays a major role in the metabolism and storage of nutrients, synthesis of various proteins and coagulation factors, as well as detoxifying many metabolic by-products (1).

Embryology of the liver

In the human embryo, the liver is derived from the endoderm, one of the three germ layers formed from gastrulation (2). It first appears as a hollow endodermal bud derived from the foregut. Hepatoblasts, the progenitor of liver cells, derived entirely from this budding endoderm, are identifiable from about 28 days (2). The vascular sinusoidal network of the embryonic liver is derived from the mesodermal layer. Bile ducts are derived from two distinct elements. Intrahepatic bile ducts appear from about seven weeks while the extrahepatic bile ducts are derived from the budding foregut endoderm (2). The weight of the liver of a newborn is approximately 200g, ~ 5% of the body mass. This is very small compared to the liver of a full grown adult which weighs approximately 1.2-1.8 kg (2).

The main driving force in the generation of bile flow in early life is the hepatocyte secretion of bile acids. There are evidences to suggest that there is a period of 'physiological cholestasis' associated with immature or altered metabolism and transport of bile acids at birth (3). This may be due to a combination of structural and functional immaturity of the hepatic excretory functions in the newborns (4).

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Neonatal cholestasis

The response of the foetal and newborn liver to insult and injury is limited. This is because the ability of the closely interdependent structures of a developing liver, i.e. hepatocytes, small intrahepatic bile ductules, large extrahepatic bile ducts, of expressing in the face of a variety of insults is limited as well (5). Injury to the newborn liver usually manifests as neonatal cholestasis. Clinically, this manifests as variable degree of pale stools and enlarged liver, and biochemically as prolonged conjugated hyperbilirubinaemia (6, 7).

Causes and clinical features of neonatal cholestasis

Many causes have been identified to cause injury to the newborn liver (8, 9). These may include intrahepatic causes, which may be either sporadic or familial, and extrahepatic causes (8). In the 146 cases of neonatal cholestasis referred to the Paediatric Unit of University of Malaya Medical Centre (UMMC), Kuala Lumpur, approximately 30% of cases were eventually found to have biliary atresia (BA, Table 1) (8-10).

Most of the infants with liver injury present ascholestatic jaundice with variable degree of pale stools and enlarged liver (7). There are considerable overlap in the clinical features of various cause of neonatal cholestasis (Table 2) (7, 11). Jaundice is a universal clinical features of neonatal cholestasis (7). However, there is no evidence to suggest that infants with BA have an earlier onset or more severe degree of jaundice than those with other causes of neonatal cholestasis (11). In addition, it is also impossible to differentiate the underlying cause of neonatal cholestasis based on the size of hepatomegaly (11). Hence, it is important to realise that each of the presenting features of neonatal liver disease is an imperfect way of discerning the underlying cause of the cholestasis. It is always a challenge for paediatricians to identify the underlying cause of the insult to liver even though their clinical manifestations may be very similar (7, 12).

Importance of early diagnosis

The majority of infants with cholestatic liver disease present during the first month of life (13). Thus, prompt and efficient differentiation of cholestatic jaundice from common physiological hyperbilirubinaemia of the neonate or the prolonged jaundice occasionally associated with breast feeding is essential (14). This is because in BA, when hepatoportoenterostomy is

Table 1: *Major causes of neonatal cholestasis seen at the University of Malaya Medical Centre, Kuala Lumpur; 1996 – 2004*

Underlying causes	N	%
Bile duct obstruction		
Biliary atresia	42	29
Other causes	4	3
Infections		
Idiopathic	56	38
Cytomegalovirus	13	9
Other infections	5	3
Progressive familial intrahepatic cholestasis	5	3
Endocrine causes	5	3
Metabolic causes	3	2
Acute liver failure	4	3
Parenteral nutrition-related cholestasis	7	5
Perinatal asphyxia	2	1
Total (%)	**146**	**100**

Adapted from: Lee WS, *et al*. Aetiology and outcome of neonatal cholestasis in Malaysia. *Singapore Med J* 2010; 51: 434-439.

Table 2: *Clinical features of neonatal cholestasis seen at the University of Malaya Medical Centre, Kuala Lumpur; 1996 – 2004*

	All causes (n=146) N (%)	Biliary atresia (n=35) N (%)	Other diagnoses (n=111) N (%)
Jaundice	146 (100)	35 (100)	111 (100)
Enlarged liver	139 (95)	35 (100)	104 (94)
Enlarged spleen	76 (52)	18 (51)	58 (52)
Colour of stools			
Pigmented	44 (30)	2 (6)	42 (38)
Slightly pale	33 (22)	4 (11)	29 (26)
Pale	69 (47)	29 (83)	40 (36)

Adapted from: Lee WS, *et al*. Clinical features differentiating biliary atresia from other causes of neonatal cholestasis. *Annals Acad Med Singapore* 2010; 39: 648-654.

Table 3: *Factors contributing to delayed referral in 65 infants with neonatal cholestasis seen at University of Malaya Medical Centre, Kuala Lumpur*

Factors	n	%
Repeated reassurances by primary health care staffs	17	26
Failure of medical services at referring hospital	7	11
No actions on blood investigations	1	
Delayed review of nuclear medicine scan	1	
Failed percutaneous cholangiogram	1	
Inconclusive liver biopsy	1	
Liver biopsy results unavailable for review	1	
Incorrect diagnosis	2	
Reluctance of parents for referral	5	8
Appropriate action / referral	8	55

Adapted from: Lee WS. Pre-admission consultation and late referral in infants with neonatal cholestasis. *J Paediatr Child Health* 2008; 44: 57-61.

performed before 60 days of age, the percentage of infants with bile flow is between 67% and 82% (15, 16). This is reduced to 45-62% if the surgery is performed after 60 days (15, 16). However, a delay in the referral of infants with neonatal cholestasis for appropriate investigations and management remains common (12, 17).

Reasons for delayed referral and diagnosis

Failure to realise this overlapping clinical features has often lead to delayed referral, diagnosis and appropriate management (Table 3) (12). In UMMC, the median age of referral for infants with neonatal cholestasis was 59 days (7). Factors contributing to a delay in referral included repeated false reassurance about the benign nature of neonatal cholestasis by medical and para-medical staffs, failure of hospital services, and parental refusal for referral (12).

Liver histology in neonatal cholestasis

Many biochemical and radiological methods are available to differentiate obstructive from non-obstructive causes of neonatal cholestasis, though none are perfect (11). Histological examination of the biopsy materials obtained from infants with neonatal cholestasis has been found to be highly specific and sensitive in differentiating BA from other non-obstructing causes (11, 18). Liver biopsy can

be performed safely even in the smallest of infants (11, 18). Bile ductular proliferation, bile plugging, multinucleated giant cells, focal necrosis of the parenchyma, extramedullary haemopoiesis and inflammatory cell infiltrate in the portal area are all well-recognised histological features of BA (18). Most of these features are more commonly seen in older infants with BA with advanced stages of biliary cirrhosis (19).

However, it should be remembered that the histological features of biliary atresia is dynamic. There remains considerable overlap in the histological features of BA and other non-obstructive causes of neonatal cholestasis (Table 4) (20). Early in life, characteristic features of obstruction may not be obvious (Figures 1 & 2) (20). The staining of the liver biopsy tissue with a special stain for biliary epithelium helps to make an accurate diagnosis of biliary atresia (Figure 3) (21).

Scoring system in the interpretation of histology in neonatal cholestasis

Because of the non-specific nature of the histology in cholestatic neonatal liver disease, many authors

Table 4: *Histological features of neonatal cholestasis (Brough & Bernstein, 1969)*

Biliary atresia (n=58)	
Major features	Bile ductular proliferation (100%)
Minor features	Portal fibrosis (47%)
	Bile plugs in dilated ducts (40%)
	Giant cell transformation (25%)
	Portal and/or lobular inflammatory infiltrate (28%)
Neonatal hepatitis (n=29)	
Major features	Hepatocellular damage and hepatitis (100%)
Minor features	Giant cell transformation (variable)
	Bile ductular proliferation (14%)*
	Bile stasis (almost exclusively intracellular and canalicular)
	Portal fibrosis – slight

* Bile ductular proliferation was generally mild and was focally or irregularly distributed. In addition, the distortion and irregularity of the proliferating ducts, characteristically seen in biliary atresia, were lacking.

Adapted from: Brough AJ and Bernstein J. Liver biopsy in the diagnosis of infantile obstructive jaundice. *Pediatrics* 1969; 43: 519-526.

Table 5: *Outcome of 57 children with biliary atresia referred to University Malaya Medical Centre, Kuala Lumpur; 1996 – 2004*

	n	%
Alive, no morbidity	16	28
Alive, major morbidity	5	9
Alive, liver transplant	2	4
Died, without surgery	9	16
Died, unsuccessful surgery	23	40
Died, after liver transplant	2	4
Total	57	100

Adapted from: Lee WS, *et al*. Outcome of biliary atresia in Malaysia. *J Paediatr Child Health* 2009.

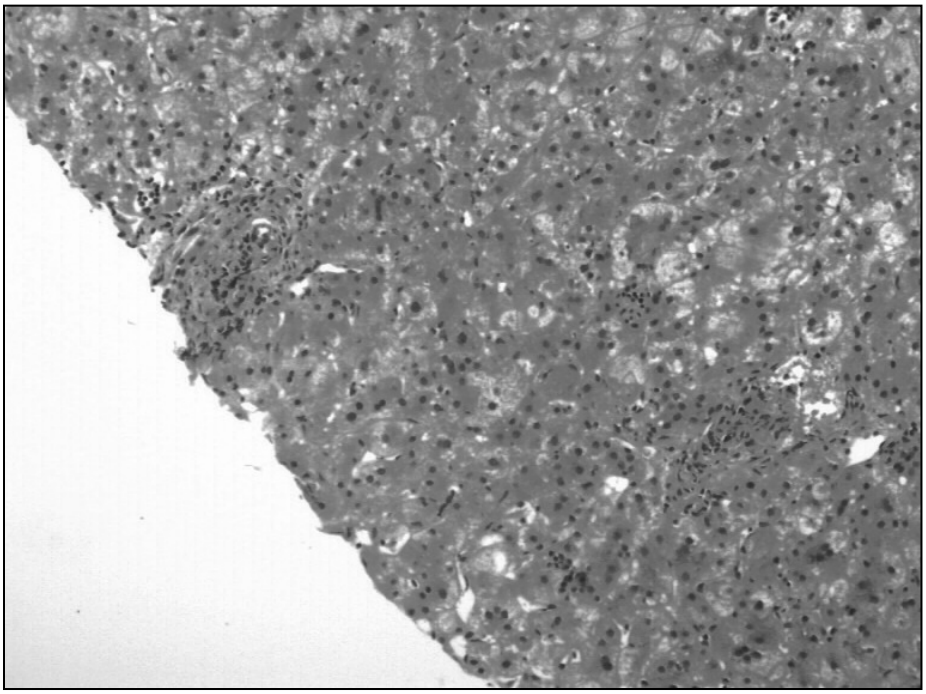

Figure 1: *Liver biopsy of a patient with biliary atresia obtained at 34 days of age*

A portal tract was shown here with no obvious bile ductular proliferation. There was hepatocytic degeneration and swelling (H&E, x 200).

Adapted from: Lee WS, *et al*. Diagnostic usefulness of a 7-feature, 15-point scoring system in the interpretation of liver histology in neonatal cholestasis. *World J Gastroenterol* 2009.

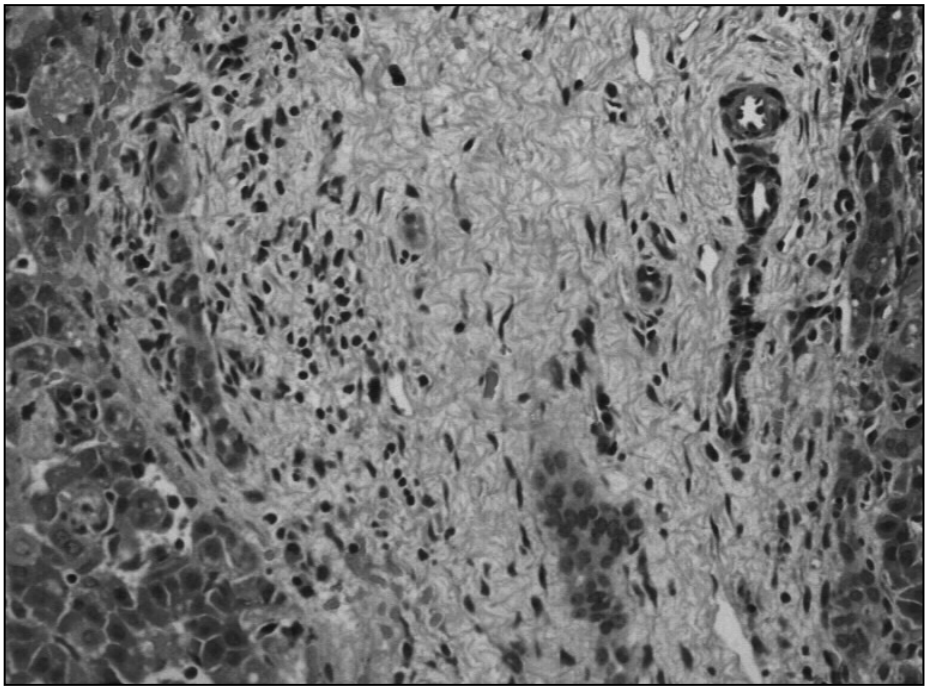

Figure 2: *Liver biopsy of the same patient obtained at 45 days of age*

A portal tract was shown here with marked bile ductular proliferation, and bile plug in bile ductules, typical of biliary atresia (H&E, x 200).

Adapted from: Lee WS, *et al*. Diagnostic usefulness of a 7-feature, 15-point scoring system in the interpretation of liver histology in neonatal cholestasis. *World J Gastroenterol* 2009.

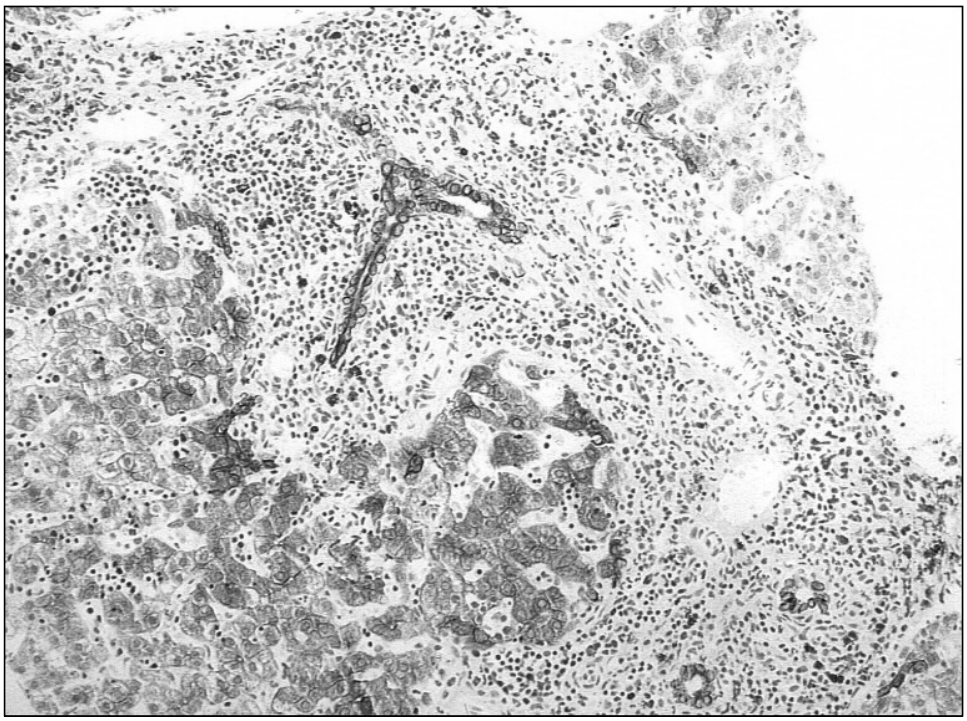

Figure 3: *Liver biopsy stained with MnF116 in biliary atresia showing a portal tract with marked bile ductular proliferation*

Marked bile ductular proliferation with tortuosity within a portal tract (MnF116, x 200).

Adapted from: Lee WS, *et al*. Diagnostic usefulness of a 7-feature, 15-point scoring system in the interpretation of liver histology in neonatal cholestasis. *World J Gastroenterol* 2009.

have attempted to devise various histological scoring systems. The scoring system devised by Zerbini *et al* suffered from being too complex (22), and has not been adopted widely.

A histology scoring system was devised at the Department of Paediatrics, UMMC, based on 7-feature (portal ductal proliferation, bile plugs in the ductules, porto-portal bridging, lymphocytic infiltration in the portal region, multinucleated hepatocytes, neutrophilic infiltration, hepatocellular swelling), 15-scoring (0-15) scoring system (21). With this system, Lee and Looi reviewed blindly 84 liver biopsy specimens obtained from 78 infants with neonatal cholestasis. A score of 7 was found to have the best overall diagnostic utility to differentiate BA from other intrahepatic cholestasis histologically (sensitivity for BA 88%, specificity 94% and accuracy 92%) (21).

Biliary atresia

BA is an idiopathic condition characterised by progressive fibrosing obliteration of both intra- and extrahepatic bile ducts (23). It is the most important cause of neonatal cholestasis worldwide, including Malaysia (10, 23). It is also the most important indication for childhood liver transplantation the world over (24). The main challenges facing infants and children with BA in Malaysia is late surgery leading to a poor outcome (Table 5) (10, 12).

Outcome of biliary atresia in UMMC, Malaysia

The outcome of infants with BA with unsuccessful surgery is poor (23). Without liver transplantation, the median age of survival was 15 months (23). In those with unsuccessful surgery, liver transplantation is essential to ensure long term survival. In the United Kingdom, where surgery for BA is centralized to three regional centres, the 4-year actuarial survival (with liver transplantation) was 89%, while the 4-year actuarial survival with native liver was 51% (25). In UMMC, a study involving 57 infants with BA from 1996 to 2005 showed that the 2-year actuarial survival (with liver transplantation) was 40%, while the 2-year actuarial survival with native liver was 37% (10).

Table 6: *Outcome of biliary atresia in UMMC (1996-2004) as compared to other reported series in the world.*

Centre	Survival rate with native liver (%)	Survival rate with native liver and liver transplant (%)
Japan	60	75
Taiwan	35	42
England and Wales	51	89
United States	56	91
France	40	74
Canada	33	85
Switzerland	37	92
Malaysia (UMMC)	37	40

Adapted from: Lee WS, *et al*. Outcome of biliary atresia in Malaysia. *J Paediatr Child Health* 2009.

Two factors contributed to the poorer surgical outcome and actuarial survival for BA in UMMC as compared to other more advanced countries in the world. They are a delay in referral for infants with neonatal cholestasis for appropriate diagnosis and surgery for BA, as well as limited availability of liver transplantation in children who have end stage liver failure in Malaysia (10). Nevertheless, the survival rate with native liver in children with BA operated at UMMC is comparable to other countries in the world (Table 6) (10).

Future challenges

The challenges of neonatal cholestasis and BA in Malaysia are manifold. Firstly, health care providers need to be aware of the potentially serious nature of an infant with prolonged cholestatic jaundice and pale stools. Obstructive causes need to be excluded in the first instance. Secondly, infants suspected of having BA should be managed in a centre with appropriate medical and surgical expertise. Finally, liver transplantation service in Malaysia should be expanded so that more children with end stage liver failure can receive life-saving surgery.

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