Iranian Red Crescent Medical Journal

HYPOTHESIS

Can Stereological Studies Be Helpful in Differentiating Biliary Atresia from Neonatal Hepatitis?

A Khosravi[1, 2], MH Anbardar[1], AH Hosseini[1], MH Imanieh[3*], A Noorafshan[4]

[1]Faculty of Medicine, [2]Student Research Lab, [3]Gastrohepatology Research Center, Department of Pediatrics, [4]Department of Anatomy, School of medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

Cholestatic jaundice is a potentially dangerous condition which is often misdiagnosed by paediatricians as physiological or breast milk jaundice. The two most common causes of neonatal cholestasis (NC) are biliary atresia (BA) and neonatal hepatitis (NH). Early and accurate differentiation of these two entities is very important as early surgery in BA improves the biliary drainage but the delay leads to irreversible hepatocellular damage. There has been much discussion over the value of hepatobiliary radioisotopic scans, liver histopathological features, serum g-glutamyl transpeptidase (GGTP) levels, and other tests which are widely used for differentiation of BA from NH. Stereology provides practical techniques for extracting quantitative information about a three-dimensional material from measurements made on two-dimensional planar sections of the tissues. We suggest that the stereological study of different components of liver tissue may be useful in determining the function of liver and differentiating some liver diseases such as biliary atresia and neonatal hepatitis.

Keywords: Cholestasis; Biliary atresia; Neonatal Hepaatitis; Stereology; Liver biopsy

Biliary Atresia and Neonatal Hepatitis

Neonatal jaundice occurs in 10–15% of neonates and is usually physiological or due to breast milk jaundice. It is often benign. Cholestatic jaundice, on the other hand, is relatively less common though potentially dangerous and is often misdiagnosed by paediatricians as physiological or breast milk jaundice.[1] The incidence of neonatal cholestasis (NC) is around 1 in 2500 live births in the West;[2,3] in India it constitutes 30% of all hepatobiliary disorders.[4]

The two most common causes of neonatal cholestasis (NC) are biliary atresia (BA) and neonatal hepatitis (NH).[1] Biliary atresia (BA), a condition unique to infancy, is the end result of a destructive idiopathic and inflammatory process affecting both the intrahepatic and extrahepatic ducts, leading to obstruction of the biliary tract and biliary cirrhosis.[5] The incidence of BA is approximately 1 in 8000 to 1 in 15000 live births and in developing countries it constitutes 25.8–34% of all NC cases.[6]

Early and accurate differentiation of these two entities, BA and NH, is very important as early intervention in the form of Kasai portoenterostomy in BA improves the biliary drainage and prognosis.[7,8] Surgery, therefore, in BA has to be performed at an earliest opportunity preferably within first 8 weeks of life without loss of time,[9] but delay leads to irreversible hepatocellular damage and poor long-term survival.[7]

In 70% of cases, differentiation between BA and NH is a working diagnosis.[9,10] Operative cholangiography is the gold standard to differentiate between BA and NH,[11] but its many disadvantages, i.e. its invasive nature, potential surgical hazards, requirement for hospitalization, expertise and consequent expenses, have led to the search for a simpler and less cumbersome diagnostic approach.[12] Many tests have been used for reaching a diagnosis before subjecting these babies to the operation:

Hepatobiliary radioisotopic scans (hepatobiliary iminodiacetic acid) have been widely used for this purpose. However, hepatobiliary iminodiacetic acid scan is time-consuming as the priming of the patient with ursodeoxycholic acid or phenobarbitone is required and most importantly only excretion of

***Correspondence:** Mohammad Hadi Imanieh, MD, Gastrohepatology Research Center, Department of Pediatrics, Shiraz University of Medical Sciences, Shiraz, Iran. Tel: +98-711+6474263, Fax: +98-711-6474263, e-mail: imaniehm@sums.ac.ir

Received: November 12, 2009 Accepted: January 25, 2010

radioisotope in the duodenum rules out BA. Thus, non-excretion of radioisotope neither confirms nor rules out the diagnosis of BA.[13] The test has also been impeded by a high rate of false positive results.[14,15]

There has been much discussion about the value of liver biopsy in the diagnosis of BA.[13] Some studies have emphasized upon the usefulness of liver histopathological features,[16,17] while others have pointed out the lack of reliability of the histological diagnosis based on liver biopsy specimens.[18] There is some disagreement among different authors concerning histopathological findings which could discriminate between intrahepatic and extrahepatic causes of NC.[13] Morphological alterations in BA are closely similar to and often indistinguishable from those of NH. Thus, the biggest challenge in histological diagnosis of diseases causing NC is the differentiation between BA and NH.[19]

Serum g-glutamyl transpeptidase (GGTP) levels are raised in numerous hepatic and extrahepatic conditions, including neonatal cholestasis.[20] In isolation, this test consistently had high sensitivity for BA, but the reported specificity was as low as 33% depending on the cut-off level taken.[21] Thus, independently, this test has no clear advantage in overcoming the problem of false positive results.[12]As mentioned above, it is not an easy task to differentiate BA from NH and studies for searching and evaluating of different tests and diagnostic approaches should be continued.

Stereological Studies

Stereological studies are more and more frequent in literature, particularly in the development/evolution, kidney pathology, and neurosciences areas. Stereologic methods are practical tools based on sound mathematical and statistical principles.[22]
Stereology provides practical techniques for extracting quantitative information about a three-dimensional material from measurements made on two-dimensional planar sections of the tissues. It is an important and efficient tool in many applications of microscopy and biosciences including histology.[23]

Hypothesis

It is not an easy task to differentiate biliary atresia from neonatal hepatitis in neonatal cholestasis. Many tests have been used to get a diagnosis before subjecting the infants to laparotomy but none of them have high sensitivity and specificity.[1]

Although many studies have pointed out the lack of reliability of the histological diagnosis based on liver biopsy specimens,[18] some have emphasized that liver histological examination is the most reliable single test for the differential diagnosis[24] or they have stressed the usefulness of liver histopathological features.[16,17] A study also showed that quantitative analysis of proliferating ductuli and proliferation activity of ductal epithelial cells might be helpful in differential diagnosis of neonatal hepatitis and biliary atresia.[25]

On the other hand, quantification of number, size, and distribution of nephrons, cells, and other components yield important information about the function and organization of the kidney and their reaction to trauma, chemicals, and disease.[22]

Therefore, stereological study of different components of liver tissue (including stereological assessment of the volume of the hepatocytes, volume of the hepatocyte nuclei, number of hepatocytes per unit volume, length of biliary ducts per unit volume, biliary ducts diameter seen at the biliary plates, biliary ducts wall thickness, and others) may also be useful in determining the function of liver and differentiating some liver diseases such as biliary atresia and neonatal hepatitis.

Evaluation of the Hypothesis

Our Hypothesis can be evaluated by retrospective studies on babies with bialiary atresia who are diagnosed after operative cholangiography and neonatal hepatitis. The liver biopsies taken before the surgery from all of the infants in both groups can be evaluated. We can assess these parameters by stereological methods: volume of the hepatocytes, volume of hepatocyte nuclei, number of hepatocytes per unit volume of the tissue, biliary ducts diameter seen at the biliary plates, biliary ducts wall thickness, length of biliary ducts per unit volume of the tissue, volume density of the fibrotic tissue and other important components. Finally, the gathered data for these two groups can be analyzed by using non-parametric tests to show if they can be helpful in differentiating biliary atresia from neonatal hepatitis.

We have designed a study using these techniques to support our hypothesis. However, it is better to design more studies to prove that stereological stud-

ies can be helpful in differentiating these two or other liver diseases.

Conflict of interest: None declared.

References

1 Poddar U, Thapa BR, Das A, Bhattacharya A, Rao KL, Singh K. Neonatal cholestasis: differentiation of biliary atresia from neonatal hepatitis in a developing country. *Acta Paediatr* 2009;**98**:1260-4. [19469771] [doi:10.1111/j.1651-2227.2009.01338.x]

2 Suchy FJ. Approach to the infant with cholestasis. In: Suchy FJ, Sokol RJ, Balistreri WF, eds. Liver Disease in Children, 2nd edn. Philadelphia, PA: Lippincott Williams & Wilkins; 2001: p. 187–94.

3 McKiernan PJ. Neonatal cholestasis. *Semin Neonatol* 2002;**7**:153-65. [12208100] [doi:10.1053/siny.2002.0103]

4 Indian Academy of Pediatrics. Pediatric Gastroenterology Subspecialty Chapter. Consensus report on neonatal cholestasis syndrome. Pediatric Gastroenterology Subspecialty Chapter of Indian Academy of Pediatrics. *Indian Pediatr* 2000;**37**:845-51. [10951633]

5 Mieli-Vergani G, Vergani D. Biliary atresia. Semin Immunopathology, 2009

6 Bazlul Karim AS, Kamal M. Cholestatic jaundice during infancy: experience at a tertiary-care center in Bangladesh. *Indian J Gastroenterol* 2005;**24**:52-4. [15879649]

7 Mieli-Vergani G, Howard ER, Portman B, Mowat AP. Late referral for biliary atresia--missed opportunities for effective surgery. *Lancet* 1989;**1**:421-3. [2563796] [doi:10.1016/S0140-6736(89)90012-3]

8 Miyano T, Fujimoto T, Ohya T, Shimomura H. Current concept of the treatment of biliary atresia. *World J Surg* 1993;**17**:332-6. [8337879] [doi:10.1007/BF01658700]

9 Petersen C. Pathogenesis and treatment opportunities for biliary atresia. *Clin Liver Dis* 2006;**10**:73-88. [16376795] [doi:10.1016/j.cld.2005.10.001]

10 A-Kader HH, Balistreri WF. Neonatal hepatobiliary disease. *Semin Gastrointest Dis* 1994;**5**:65-77. [8049908]

11 Ohi R, Hanamatsu M, Mochizuki I, Chiba T, Kasai M. Progress in the treatment of biliary atresia. *World J Surg* 1985;**9**:285-93. [3993060] [doi:10.1007/BF01656322]

12 Arora NK, Kohli R, Gupta DK, Bal CS, Gupta AK and Gupta SD. Hepatic technetium-99m-mebrofenin iminodiacetate scans and serum g-glutamyl transpeptidase levels interpreted in series to differentiate between extrahepatic biliary atresia and neonatal hepatitis. Acta Paediatr 2001; 90: 975-81.

13 Rastogi A, Krishnani N, Yachha SK, Khanna V, Poddar U, Lal R. Histopathological features and accuracy for diagnosing biliary atresia by prelaparotomy liver biopsy in developing countries. *J Gastroenterol Hepatol* 2009;**24**:97-102. [19196397] [doi:10.1111/j.1440-1746.2008.05737.x]

14 Spivak W, Sarkar S, Winter D, Glassman M, Donlon E, Tucker KJ. Diagnostic utility of hepatobiliary scintigraphy with 99mTc-DISIDA in neonatal cholestasis. *J Pediatr* 1987;**110**:855-61. [3585600] [doi:10.1016/S0022-3476(87)80396-7]

15 Johnson K, Alton HM, Chapman S. Evaluation of mebrofenin hepatoscintigraphy in neonatal-onset jaundice. *Pediatr Radiol* 1998;**28**:937-41. [9880637] [doi:10.1007/s002470050505]

16 Yachha SK, Mohindra S. Neonatal cholestasis syndrome: Indian scene. *Indian J Pediatr* 1999;**66**:S94-6. [11132478]

17 Dehghani SM, Haghighat M, Imanieh MH, Geramizadeh B. Comparison of different diagnostic methods in infants with cholestasis. *World J Gastroenterol* 2006;**12**:5893-6. [17007060]

18 Landing BH. Considerations of the pathogenesis of neonatal hepatitis, biliary atresia and choledochal cyst: the concept of infantile obstructive cholangiopathy. In: Bill AH, Kasai M, eds. Progress in Pediatric Surgery, Vol. 6. Baltimore, MD: University Park Press; 1974: p. 113-39.

19 Nayak NC, Vasdev N. Neonatal cholestasis syndrome: identifying the disease from liver biopsy. *Indian Pediatr* 2002;**39**:421-5. [12037270]

20 Radhakrishnan T, Paramananda-Rao K, Srinivasan NG. Serum gamma-glutamyl transpeptidase activity in diseases of the hepatobiliary system. *Indian J Exp Biol* 1976;**14**:254-7. [11182]

21 Manolaki AG, Larcher VF, Mowat AP, Barrett JJ, Portmann B, Howard ER. The prelaparotomy diagnosis of extrahepatic biliary atresia. *Arch Dis Child* 1983;**58**:591-4. [6137197] [doi:10.1136/adc.58.8.591]

22 Nyengaard JR. Stereologic methods and their application in kidney research. *J Am Soc Nephrol* 1999;**10**:1100-23. [10232698]

23 Mandarim-de-Lacerda CA. Stereological tools in biomedical research. *An Acad Bras Cienc* 2003;**75**:469-86. [14605681] [doi:10.1590/S0001-37652003000400006]

24 Lai MW, Chang MH, Hsu SC, Hsu HC, Su CT, Kao CL, Lee CY. Differential diagnosis of extrahepatic biliary atresia from neonatal hepatitis: a prospective study. *J Pediatr Gastroenterol Nutr* 1994;**18**:121-7. [8014758] [doi:10.1097/00005176-199402000-00001]

25 **25** Aktas S, Diniz G, Ortac R. Quantitative analysis of ductus proliferation, proliferative activity, Kupffer cell proliferation and angiogenesis in differential diagnosis of biliary atresia and neonatal hepatitis. *Hepatogastroenterology* 2003;**50**:1811-3. [14696411]