

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

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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 Hepatitis; 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

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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 diagnosis²⁴ 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 biliary 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.

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