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EARLY DIAGNOSTIC DIFFERENTIATION IN NEONATAL CHOLESTASIS TO OPTIMIZE THERAPEUTIC OUTCOMES

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ABSTRACT

Neonatal cholestasis represents a category of diseases which frequently manifest with comparable clinical symptoms, and it is difficult to provide their precise diagnosis, yet it is crucial to do so to ensure adequate management. It is important to distinguish these conditions by the study of liver biopsy samples to influence adequate treatment approaches. We assessed the clinical features, laboratory results, and histopathological features of pretreatment liver biopsy specimens of infants with biliary atresia (n=66), intrahepatic bile duct paucity (n=15) and neonatal hepatitis (n=21) in the current study. Gender distribution was almost even in patients with biliary atresia and intrahepatic bile duct paucity but the neonatal hepatitis group showed the presence of more male infants. Of all the laboratory parameters investigated, the levels of only one enzyme, gamma-glutamyl transferase (GGT), differed significantly between the groups, indicating its possible value as a parameter helping to distinguish between these conditions. They found significant histopathological characteristics which can be helpful in differentiating these two bile duct diseases. Cases of biliary atresia had marked bile ductular proliferation and severe fibrosis, whereas the degree of bile duct loss differed among the groups. In particular, the mean portal tracts with bile duct loss were significantly greater in cases of intrahepatic bile duct paucity (about 74%) than those of neonatal hepatitis (about 39%) and the difference was of strong statistical significance ($p < 0.001$). These data underline that bile ductular proliferation, loss of bile ducts and advanced fibrosis are key findings helping to distinguish between neonatal cholestatic diseases. Due to the fact that loss of bile ducts is also frequently observed in infants with neonatal hepatitis, it is advisable to apply a higher bar during the diagnostics. Specifically, the diagnosis of intrahepatic bile duct paucity ought to entail the loss of bile duct in over two-thirds of portal tracts to prevent false diagnosis. To conclude, meticulous histological evaluation of the level of bile ductular proliferation, the extent of bile duct loss and the stage of fibrosis can give useful data in differentiating between biliary atresia, intrahepatic bile duct paucity, and neonatal hepatitis. It is necessary to implement more stringent definitions of bile duct loss to make an accurate diagnosis of intrahepatic bile duct paucity, which will enhance the making of proper treatment decisions and outcomes in infants with neonatal cholestasis.

Keywords: Neonatal cholestasis, Biliary atresia, Intrahepatic bile duct paucity, Liver biopsy, Bile duct loss

INTRODUCTION

Neonatal cholestasis is an infant condition that is characterized by the continuance of jaundice, dark-colored urine, and pale or clay-colored stool past the age of two to three weeks. The condition entails a vast variety of diseases, which can be classified broadly as obstructive and non-obstructive

causes. The obstructive causes include biliary atresia, common bile duct obstruction, choledochal cyst with biliary sludge, and impacted thick bile or mucous plugs. The non-obstructive forms are bacterial and viral infections, metabolic disorders, intra hepatic bile ducts paucity, cirrhosis, long term parenteral nutrition, adverse drug reactions, and idiopathic neonatal hepatitis [1,2]. The severity of physiological cholestasis presentation may substantially differ among infants, which commonly results in a delayed diagnosis during the first three to four months of life. The appropriate differentiation of biliary atresia and its distinction among other causes is of utmost importance especially since it directly impacts the timing of surgical intervention which can impact the outcomes significantly. The combination of laboratory tests, imaging and liver biopsy usually allows to achieve the correct diagnosis, although the latter is the most informative and the definitive method of detection that may be used. The typical features of histopathological examination of liver tissue in biliary atresia include cholestasis, portal tracts edematous fibrosis, and periportal ductular proliferation, giant cell transformation of hepatocytes, and acute and chronic inflammation in the portal and periportal regions [3]. The following findings are not specific to biliary atresia though and are largely shared with neonatal hepatitis. Several findings are typical of liver biopsy specimen of infants with neonatal hepatitis such as cholestasis, giant cell transformation, both lobular and portal inflammation, progressive fibrosis, ballooning degeneration of hepatocytes and extramedullary hematopoiesis. Due to considerable degree of histological overlap, i.e., different extents of cholestasis, giant cell transformation, and portal inflammation with increasing fibrosis, differentiation between biliary atresia and neonatal hepatitis has been found to be difficult even to the experienced pathologists [4]. Clinical and laboratory features mimic each other and make a definite diagnosis even more challenging to arrive at, which explains the need to pay closer attention to subtle histological signs and match them with clinical and biochemical evidence. The purpose of the study is to examine both histological aspects and clinical parameters on liver biopsy samples in order to identify what markers could help to distinguish between neonatal cholestasis etiologies with greater accuracy. It is hoped that by delving into the details of certain tissue attributes and their connection to clinic manifestations, it would be possible to increase the accuracy of the diagnosis and, by extension, direct more timely and corrective therapeutic processes [5]. Enhancing the distinction between biliary atresia and the rest of the causes of neonatal cholestasis is important, as it determines the line of management, prognosis, and eventually survival and quality of life of the infected infants.

MATERIALS AND METHODOLOGY

This study involved the selection of liver biopsy specimens from a total of 112 patients who presented with neonatal cholestasis. The final clinicopathological diagnoses of these patients included a range of etiologies, with biliary atresia being the most common diagnosis, accounting for 66 cases (58.9%). Other diagnoses included intrahepatic bile duct paucity, which was further divided into syndromic (9 patients, 8.0%) and non-syndromic forms (12 patients, 10.7%), and neonatal hepatitis (15 patients, 13.4%) [1]. Additional cases involved less frequent conditions such as progressive familial intrahepatic cholestasis (PFIC), arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome, and cholestasis related to total parenteral nutrition (TPN), collectively representing 10 patients The clinical presentations, laboratory findings, hepatobiliary imaging results, and histopathological features of pretreatment liver biopsy specimens were thoroughly evaluated for 66 patients diagnosed with biliary atresia, 21 patients with intrahepatic bile duct paucity, and 15 patients with neonatal hepatitis [2]. The majority of the patients underwent a single pretreatment liver biopsy, with the exception of six patients from whom two biopsy specimens were obtained at different times. Liver tissue samples were formalin-fixed and embedded in paraffin blocks for microscopic examination. Sections were stained using hematoxylin and eosin (H&E) to evaluate cellular and tissue morphology, as well as Masson's trichrome stain to assess the extent of fibrosis. Immunohistochemical staining was also performed using antibodies against cytokeratin 7 (CK7), a marker for bile duct epithelium, at a dilution of 1:200 [3]. This staining aided in the identification and evaluation of bile ductular structures within the portal tracts Histopathological

examination focused on key hepatocyte changes, including giant cell transformation—characterized by multinucleated hepatocytes—and hepatocyte ballooning, which indicates cellular injury and swelling. The abnormalities of portal tract were also thoroughly evaluated and of special interest was the amount of portal inflammation, bile ductular proliferation, loss of bile ducts and the intensity of fibrosis. Also, the existence of extramedullary hematopoiesis the abnormal presence of blood cell formation outside of the bone marrow was noted [4]. The liver biopsy samples were studied in a systematic manner to determine the different histological features that may help distinguish among biliary atresia, intra hepatic bile duct paucity and neonatal hepatitis. Such a wholesome strategy enabled a correlation of clinical and laboratory observations with the histopathological changes and thus helped to achieve a more accurate diagnosis of the underlying causes of neonatal cholestasis [5]. The results of the current study are aimed at improving the diagnostic specificity and informed management approaches that should be applied to infants admitted with the case of cholestasis in the neonatal phase.

RESULTS

The study revealed that the samples of patients with biliary atresia and intrahepatic bile duct paucity almost equalized in terms of male and female patients. Conversely, there was strong male predominance in neonatal hepatitis group with a male: female ratio of approximately 2:1. Patients with neonatal hepatitis were somewhat older at the time of liver biopsy (73 +/- 29 days), compared to patients with biliary atresia and intrahepatic bile duct paucity, whose mean ages were 62 +/- 37 days and 66 +/- 26 days, respectively. Of all the biochemical parameters tested to determine the abnormality in liver functions, only gamma-glutamyl transpeptidase (GGT) revealed statistically significant variations among the groups of disease conditions. The level of GGT was significantly higher in patients with biliary atresia, where the mean concentration was 432.2 +/- 275.0 IU/L compared to the patients with neonatal hepatitis (198.0 +/- 130.0 IU/L) and intrahepatic bile duct paucity (127.6 +/- 103.5 IU/L). This observation highlights the possible value of GGT as a discriminatory laboratory parameter in the variousiation of biliary atresia and other forms of neonatal cholestasis. A hepatobiliary imaging was performed in most of the patients in every group; 62 patients with biliary atresia, 14 with neonatal hepatitis and 19 with intrahepatic bile duct paucity. In patients with biliary atresia, the typical appearance of non-visualization of the gallbladder and lack of bowel activity in the scans was seen in 61/62 patients, which confirms the obstructive nature of the condition. But lack of biliary excretion was also found in a small group of patients with neonatal hepatitis (3 cases) and intrahepatic bile duct paucity (10 cases), indicating some overlapping imaging appearance which can make clinical distinction difficult. The liver biopsy specimens were crucial in the histopathological examination that gave insights on the specifics of the disease. Another feature of intrahepatic bile duct paucity was loss of bile ducts, which was encountered in 88.2 percent of the patients with neonatal hepatitis and 39.7 percent of the patients with biliary atresia. Loss of bile ducts was determined as the absence of bile ducts in portal tracts. Quantitatively, the mean percentage of portal tracts wit loss of bile ducts was maximum in intra hepatic bile duct paucity of 73.9%, neonatal hepatitis of 39.1% and minimum in biliary atresia of 7.3%, a difference which was extremely significant ($p < 0.001$). Biliary atresia was associated with periportal bile ductular proliferation which is regarded as a diagnostic indication of this disease. Whilst this proliferation was also observed in some patients with neonatal hepatitis (2 cases) and intrahepatic bile duct paucity (4 cases), it was much less pronounced than in biliary atresia, arguing against it being a merely incidental finding. Fibrosis was equally distributed in the three conditions but the intensity of advanced fibrosis, including septal fibrosis or cirrhosis, was much higher in the biliary atresia group compared with the neonatal hepatitis or intrahepatic bile duct paucity patients ($p < 0.001$). Also portal and periportal inflammation and extramedullary hematopoiesis were more common in cases of neonatal hepatitis, but again, there was no significant difference in the frequency of these changes among the three disease groups. Altogether, these findings underline that a number of histopathologic and clinical parameters may assist in differentiating the underlying

causes of neonatal cholestasis, and the variables that may be helpful diagnostic tools are specifically the loss of bile ducts, bile ductular proliferation, the extent of fibrosis, and the level of GGT.

Table 1: Comparison of Clinical, Biochemical, Imaging, and Histopathological Features in Neonatal Cholestasis Conditions.

Parameter	Biliary Atresia	Neonatal Hepatitis	Intrahepatic Bile Duct Paucity	Statistical Significance
Gender Distribution (M:F ratio)	~1:1 (Nearly equal)	2:1 (Male predominance)	~1:1 (Nearly equal)	-
Age at Liver Biopsy (days)	62 ± 37	73 ± 29	66 ± 26	-
Gamma-glutamyl Transpeptidase (GGT) (IU/L)	432.2 ± 275.0	198.0 ± 130.0	127.6 ± 103.5	Significant ($p < 0.05$)
Hepatobiliary Scan Findings	Non-visualization of gallbladder in 61/62 patients; absent bowel activity	No biliary excretion in 3 patients	No biliary excretion in 10 patients	-
Loss of Bile Ducts (%)	39.7%	88.2%	73.9%	Highly significant ($p < 0.001$)
Mean % Portal Tracts with Bile Duct Loss	7.3%	39.1%	73.9%	Highly significant ($p < 0.001$)
Periportal Bile Ductular Proliferation	Prominent (Diagnostic feature)	Present but less prominent (2 cases)	Present but less prominent (4 cases)	-
Fibrosis	Frequently observed, advanced fibrosis (septal fibrosis/cirrhosis) more common	Frequently observed, less severe fibrosis	Frequently observed, less severe fibrosis	Significant difference ($p < 0.001$)
Portal & Periportal Inflammation	Observed	More common	Observed	No significant difference
Extramedullary Hematopoiesis	Observed	More common	Observed	No significant difference

Figure 1: Hepatic Conditions Clinical Comparison, Comparative analysis of Biliary Atresia, Neonatal Hepatitis, and Intrahepatic Bile Duct Paucity

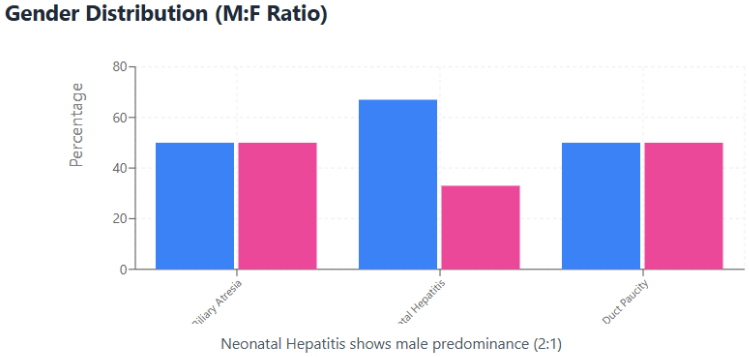
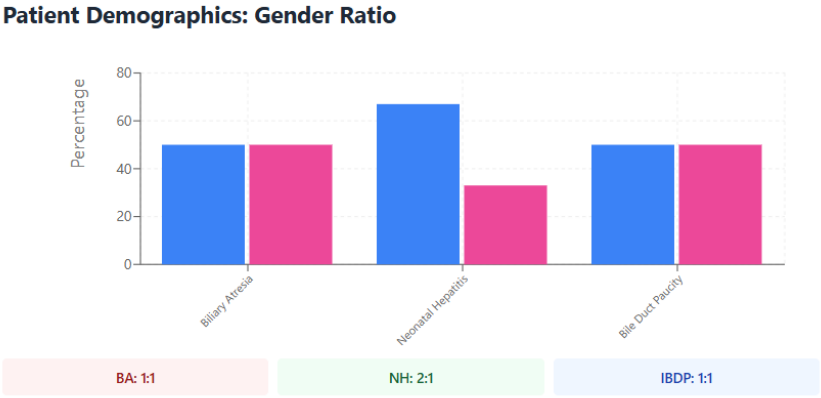


Table 2: Summary of Key Diagnostic Features in Neonatal Cholestasis Subtypes.

Feature	Biliary Atresia	Neonatal Hepatitis	Intrahepatic Bile Duct Paucity	Significance
Patient Gender Ratio (M:F)	Approximately equal	Male predominance (2:1)	Approximately equal	—
Average Age at Biopsy (days)	62 ± 37	73 ± 29	66 ± 26	—
Mean GGT Level (IU/L)	Elevated (432.2 ± 275.0)	Moderately elevated (198.0 ± 130.0)	Lower (127.6 ± 103.5)	Statistically significant ($p < 0.05$)
Hepatobiliary Scan Results	Non-visualization of gallbladder (98% cases), no bowel activity	Occasional absence of biliary excretion (3 cases)	Occasional absence of biliary excretion (10 cases)	—
Frequency of Bile Duct Loss (%)	39.7%	88.2%	73.9%	Highly significant ($p < 0.001$)
Average Portal Tracts with Bile Duct Loss (%)	7.3%	39.1%	73.9%	Highly significant ($p < 0.001$)
Presence of Bile Ductular Proliferation	Marked (Diagnostic)	Mild/moderate (2 cases)	Mild/moderate (4 cases)	—
Degree of Fibrosis	Frequent, often advanced fibrosis	Frequent, less advanced fibrosis	Frequent, less advanced fibrosis	Significant difference ($p < 0.001$)
Portal and Periportal Inflammation	Present	More common	Present	No significant difference
Extramedullary Hematopoiesis	Present	More common	Present	No significant difference

Figure 2: Hepatic Conditions: Comprehensive Clinical Comparison, Detailed analysis of Biliary Atresia, Neonatal Hepatitis, and Intrahepatic Bile Duct Paucity with statistical significance



DISCUSSION

The study offers valuable insights into the diagnostic challenges and distinguishing features of neonatal cholestasis caused by biliary atresia, neonatal hepatitis, and intrahepatic bile duct paucity. The conditions tend to overlap in their clinical and histological findings, and correct and prompt diagnosis is essential to initiation of treatment and better patient outcome One of the important observations is the almost equal gender distribution of patients with biliary atresia and intrahepatic bile duct paucity compared with severe male predominance in the cases of neonatal hepatitis. This gender difference in neonatal hepatitis possibly indicates gender differences in neonatal hepatitis susceptibility or exposure to infectious or inflammatory causes which needs further exploration. The age at biopsy was slightly older in neonates with hepatitis than those with biliary atresia and bile duct paucity and may represent the natural history or delays in clinical suspicion. Earlier biopsy in biliary atresia patients is critical, given the progressive nature of the disease and the importance of early surgical intervention. This underlines the clinical imperative for prompt recognition and referral of cholestatic infants. Among liver function tests, gamma-glutamyl transpeptidase (GGT) levels demonstrated the most significant variation, with markedly elevated values observed in biliary atresia patients. Elevated GGT is consistent with obstructive cholestasis and supports its utility as a valuable biochemical marker in distinguishing biliary atresia from other causes of neonatal cholestasis. However, the overlap in GGT levels among other groups indicates it should be interpreted alongside other clinical and histopathological data.Imaging studies reinforced the obstructive pathology of biliary atresia, with almost all patients exhibiting the classical findings of non-visualization of the gallbladder and absent bowel activity on hepatobiliary scans. However, a small number of patients with neonatal hepatitis and intrahepatic bile duct paucity also showed impaired biliary excretion, highlighting that imaging alone may not conclusively differentiate these entities. This overlap necessitates integration of histological findings to reach a definitive diagnosis Histopathology revealed that loss of bile ducts is a prominent feature in intrahepatic bile duct paucity but also occurs to a lesser extent in neonatal hepatitis and biliary atresia. The significantly higher percentage of portal tracts lacking bile ducts in bile duct paucity compared to other groups supports its diagnostic relevance. Meanwhile, pronounced bile ductular proliferation was characteristic of biliary atresia, serving as a useful histological hallmark. The presence of bile ductular proliferation in some cases of neonatal hepatitis and bile duct paucity, albeit less intense, underscores the complexity of interpreting these features in isolation. Fibrosis was frequently observed across all conditions, but advanced fibrosis and cirrhosis were notably more severe in biliary atresia, correlating with the disease's progressive obstructive pathology. More frequent in neonatal hepatitis were portal and periportal inflammation and extramedullary hematopoiesis, but these observations were not statistically significant between the groups, and therefore they could not be reliable indicators of diagnosis. Taken together, the study confirms the importance of the

multifaceted diagnostic method based on the combination of clinical information, biochemical markers, imaging, and thorough histopathological examination. The notable features that support biliary atresia include elevated GGT, massive bile ductular proliferation, and advanced fibrosis; whereas, massive loss of bile ducts argues more in favor of intrahepatic bile duct paucity. These similarities in the results of neonatal hepatitis보다 highlight the necessity of an interpreted approach and the possibility of the necessity of repeat or complementary assessment. Finally, the findings confirm that no specific test can be considered definite in diagnosing neonatal cholestasis, and it is necessary to pay attention to cautious correlation of all available data. diagnosis Early and correct distinction between the conditions is very important to influence correct management strategies and enhance the prognosis in the affected infants.

CONCLUSION

To conclude, neonatal cholestasis is a hard-clinical syndrome, the causes of which are various, and which, as a rule, have clinical and histopathological features of overlap. The present study highlights the paramount nature of a combination of clinical presentation, laboratory tests, radiological experiments, and extensive histopathological examination in precisely distinguishing between the major causes, including biliary atresia, intrahepatic bile duct paucity, and neonatal hepatitis. The almost balanced gender ratio in biliary atresia and intrahepatic bile duct paucity compared with predominance of males in neonatal hepatitis indicates that there may be differences in disease processes or risk factors that needs to be pursued. The markedly higher levels of gamma-glutamyl transpeptidase (GGT) in patients with biliary atresia than in the other two groups underlines its diagnostic usefulness as a biochemical parameter indicative of obstructive cholestasis. Nevertheless, as there is certain overlap in the levels of GGT regarding the various causes, it cannot be utilized alone but within the context of a wider diagnostics. Imaging studies, particularly hepatobiliary scans, provide important clues by demonstrating non-visualization of the gallbladder and absent bowel activity in biliary atresia, yet the presence of similar imaging patterns in some neonatal hepatitis and bile duct paucity cases highlights the limits of imaging alone for definitive diagnosis. Histopathological examination offers indispensable insights, with bile ductular proliferation emerging as a hallmark of biliary atresia and significant bile duct loss characteristic of intrahepatic bile duct paucity. The quantification of bile duct loss serves as a valuable discriminating feature, with the highest percentages found in bile duct paucity cases. Although fibrosis and cirrhosis are common across these conditions, advanced fibrosis is more pronounced in biliary atresia, correlating with its progressive obstructive pathology. While inflammation and extramedullary hematopoiesis were more frequently observed in neonatal hepatitis, their diagnostic specificity remains limited. Overall, these findings highlight the challenges in distinguishing neonatal cholestasis etiologies due to shared pathological features and overlapping clinical and laboratory findings. A multidisciplinary diagnostic approach, employing biochemical, radiological, and histological assessments, is essential for accurate diagnosis. Early and precise identification of the underlying cause is paramount to optimize treatment strategies, especially considering the time-sensitive nature of interventions such as surgery in biliary atresia. This comprehensive diagnostic approach ultimately enhances patient outcomes by enabling tailored management and avoiding delays in therapy. Future research focusing on novel biomarkers and improved diagnostic criteria may further refine the differentiation of neonatal cholestasis causes and improve clinical care.

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