CLINICO-RADIOLOGIC, LABORATORY, AND HISTOPATHOLOGIC PROFILE OF PATIENTS DIAGNOSED WITH NEONATAL CHOLESTASIS AT PHILIPPINE CHILDREN'S MEDICAL CENTER

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ABSTRACT

BACKGROUND: Neonatal Cholestasiswarrants early, accurate and prompt intervention and comprises a wide spectrum of differential diagnosis which present with overlapping features, thus making a diagnosis difficult.

OBJECTIVE: To evaluate the clinical and laboratory parameters that could aid to differentiate between intrahepatic and extrahepatic neonatal cholestasis.

METHODS: Retrospective and Descriptive study of Neonatal Cholestasis patients who underwent Liver Biopsy and admitted at the Philippine Children's Medical Center from January 2007 to December 2011.

RESULTS: Factors that favor an intrahepatic cause of Cholestasis are ultrasound finding of a normal gallbladder, marked degree of giant cell transformation and presence of extramedullary hematopoiesis. Factors that favor an Extrahepatic cause of Cholestasis are presence of Splenomegaly, markedly elevated GGT, and histopathology findings of Portal and Periportal Ductal proliferation, bile plugs, lesser degree of giant cell transformation, septal fibrosis and cirrhosis, portal and neoductular cholestasis, and Portal-Portal bridges.

CONCLUSION: In this study, meticulous history and physical examination aid in the diagnosis of Neonatal Cholestasis. Splenomegaly and markedly elevated serum GGT are suggestive of Biliary Atresia, and a normal Gallbladder by Ultrasound favors Neonatal Hepatitis. Although there is significant overlap of histopathologic findings of patients with neonatal cholestasis, certain parameters favor an extrahepatic over an intrahepatic process.

KEYWORDS: Clinical and Histopathologic features, Extrahepatic Cholestasis, Intrahepatic Cholestasis, Neonatal Hepatitis, Biliary Atresia.

INTRODUCTION

Neonatal cholestatic jaundice is defined as jaundice with elevated conjugated bilirubin due to an underlying hepatobiliary dysfunction. This usually occurs during the first month of life and persists for more than 10 days, and may present with choluria, hypocholic or acholic stools, hepatomegaly, and/or splenomegaly. It is the most common liver disease in infancy and affects about 1 in every 2500-5000 live newborns [(3)]. Neonatal cholestasis can be due to extrahepatic causes such as biliary atresia, common bile duct obstruction, choledochal cyst with biliary sludge, and an inspissated bile/mucous plug. Intrahepatic causes include bacterial and viral infections, metabolic diseases, genetic diseases, toxic, vascular, immune and idiopathic

intrahepatic bile duct paucity, cirrhosis, total parenteral nutrition, drugs, and idiopathic neonatal hepatitis[3].A delay in the diagnosis of Neonatal Cholestasis may cause irreversible damage to the liver leading to end stage liver failure or cirrhosis [2]. Approach to management differs for each etiology. Intrahepatic cholestasis is managed mostly by medical intervention alone, while extrahepatic cholestasis, particularly Biliary Atresia, warrants an early surgical intervention or Kasai procedure at not more than 8 weeks of life for a favorable outcome[3]. To make an accurate and definitive diagnosis, numerous laboratory tests, radiologic work-ups, and liver biopsy are necessary. However, due to a significant overlap in clinical and histopathologic presentation, differentiating neonatal hepatitis from biliary atresia is difficult especially in the early stages of the disease. A delay in the diagnosis would mean a delay in the management, which is very crucial particularly in cases of biliary atresia.

Numerous studies have tried to differentiate intrahepatic cholestasis from extrahepatic cholestasis using clinical and laboratory parameters and histopathologic variables.In Brazil, Brandao and colleagues evaluated 168 patients, using both clinical and laboratory parameters to differentiate extrahepatic causes from intrahepatic causes. In these study, birth weight and lengths, fecal hypocholia/acholia, hepatomegaly, and a 10.8 times increase in GGT were the only variables that would point to extrahepatic cholestasis. Over-all, both clinical and laboratory parameters have failed to identify the etiology of neonatal cholestasis.

In another study by Santos and colleagues, 46 liver biopsy specimens were evaluated using a discriminant analysis test. In the discrimination between intrahepatic cholestasis from extrahepatic cholestasis, the following histopathological variables were found to be most significant in decreasing order of the coefficient value of the canonical discriminant function: periportal ductal proliferation, portal ductal proliferation, portal expansion, cholestasis in neoductules, portal cholestasis, foci of myeloid metaplasia, portal-portal bridges, focal necrosis, cholestasis in canaliculi, periductal fibrosis and portal-central bridges. Of these, only foci of myeloid metaplasia would indicate intrahepatic cholestasis.

In a study done by Alagille and colleagues, histopathologic features that would indicate intrahepatic cholestasis are lobular disarray, giant cell proliferation, and hepatocellular necrosis, and minimal fibrosis, rare formation of neoductules, steatosis, and extramedullary hematopoiesis. Conversely, data that would indicate Extrahepatic cholestasis include neoductule proliferation, portal and perilobular fibrosis, bile plugs, normal lobular architecture, and little inflammatory response[3].

Lee and colleagues studied 102 infants with neonatal cholestasis using clinical manifestations, laboratory data and histopathologic features as parameters. Of these, 66 were biliary atresia cases, 21 were neonatal hepatitis cases and 15 were cases of intrahepatic bile duct paucity. The outcome of the study showed bile ductular proliferation, bile duct loss, and advanced fibrosis as useful parameters for the differential diagnosis of neonatal cholestasis. Giant cell transformation, ballooning degeneration, lobular disarray, portal inflammation and extramedullary hematopoiesis are among the features of neonatal hepatitis; however, these are also seen in other disease entities, even in biliary atresia. GGT concentration of

more than 300IU/L had a diagnostic accuracy of 85% for biliary atresia in patients less than 10 weeks old. In this study, GGT was the only reliable laboratory marker for making the diagnosis of biliary atresia.

Urganci and colleagues did a retrospective study of 70 infants diagnosed with cholestasis, with ages ranging from 15 days to 8 months, using clinical and laboratory parameters. This study showed that Biliary Atresia has an earlier onset of jaundice and acholic stools. Furthermore, total bilirubin levels, GGT and ALP 0were remarkably higher than in the groups with intrahepatic biliary hypoplasia or hepatocellular disease. They emphasized the role of scintigraphy in the diagnosis of biliary atresia as compared to other groups.

Lee and colleagues, in their prospective observational study of 146 patients with neonatal cholestasis, reported that the two most common causes of neonatal cholestasis were Biliary Atresia and Idiopathic Neonatal Hepatitis, accounting for 29% and 38% respectively. Thirty-nine patients died at the time of study, 35 of which succumbed to end-stage liver disease and 4 expired after liver transplant. Six of the 107 survivors had liver cirrhosis. The overall four year survival rate for patients with neonatal cholestasis with and without transplant were 73% and 72% respectively, while in patients with biliary atresia, the four year survival rate with and without liver transplant were 36% and 38% respectively.

This study aims to identify and determine the frequency of the different etiologies causing neonatal cholestasis among patients who underwent Liver Biopsy. Clinical presentation, Laboratory data and Histopathologic features will be assessed to classify neonatal cholestasis in two groups, intrahepatic and extrahepatic. Knowing which among the parameters would favor a diagnosis on each group will help both the clinicians and pathologists make a prompt definite diagnosis.

METHODOLOGY

This is a retrospective descriptive study of patients who underwent liver biopsy at the Philippine Children's Medical Center from January 2007 to December 2011 and diagnosed as a case of Neonatal Cholestasis. Diagnosis was based on the Final Histopathologic Diagnosis and Explorative Laparotomy Findings. According to the diagnosis, the patients were classified into two groups: Group 1 or the Intrahepatic group; and Group 2 or the Extrahepatic group. Pertinent clinical, radiologic and laboratory data were gathered. Histopathology slides were reviewed and certain histopathologic parameters were reevaluated and reassessed by a pathologist based on the presence and/or severity of these parameters. Chosen Clinical, Laboratory Data and Histopathologic parameters from each group were assessed to determine which among the parameters would differentiate both groups.

All patients clinically diagnosed with Neonatal Cholestasis who underwent liver biopsy at Philippine Children's Medical Center from January 2007 to December 2011 were the subjects of the study. The Hematoxylin and Eosin slides, Immunohistochemistry (IHC) slides with Cytokeratin and Histopathology results of each patient were gathered. IHC with Cytokeratin were done on cases where the portal tracts are obscured by inflammation. Inpatient and Outpatients charts were also reviewed. Patients were divided into two groups, under Group 1 are patients classified under Intrahepatic Cholestasis and Group 2 are patients classified under

Extrahepatic Cholestasis. The slides were reevaluated by an anatomic pathologist based on the following histopathologic parameters: bile duct proliferation, site of cholestasis (portal, neoductules, canalicular and intrahepatic), fibrosis (absent, portal, periportal, septal and cirrhosis), necrosis, ballooning degeneration, giant cell transformation, portal and lobular inflammation, and extramedullary hematopoiesis. The degree of lobular inflammation and giant cell transformation were graded based on the severity (absent to mild or moderate to severe). The pathologist who reevaluated the slides was blinded of the official result. For the clinical data, the patients age, gender, onset of jaundice, birthweight, absence and presence of pertinent signs and symptoms such as hypocholia or acholia, dark urine, hepatomegaly, splenomegaly and ascites noted on each patient were tabulated for each group. Pretreatment Clinical Laboratory Test results of AST, ALT, Total Bilirubin, Conjugated Bilirubin, ALP, GGT, INR, and Albumin were gathered for each patient and tabulated for each group. Radiologic findings of each patient were considered. Ultrasound Results and Hepatobiliary iminodiacetic scan (HIDA) results if done on the patient were noted and compared to the final result. All Clinical data, Laboratory Results, Histopathologic and Radiologic findings gathered were used as parameters differentiating extrahepatic cholestasis and intrahepatic cholestasis.

The clinical parameters, laboratory, and radiologic results were reviewed using the patient's inpatient and outpatient charts. Clinical data not indicated in the history and physical examination findings were excluded in the analysis. Likewise, laboratory results, ultrasound results, and other ancillary findings either not done or were missing from the charts were also excluded in the analysis. Therefore, the total number of patients evaluated in this study varied for each clinical, radiologic, and laboratory parameter considered.

RESULTS

Table 1. Patient Clinical Characteristic, according to group

Variable	N –px with available data	Intrahepatic (n=32)	Extrahepatic (n=39)	P	Interpretation
Gender	71				
Male		17	24	0.475	Not Significant
Female		15	15		
Age of Patient at time of biopsy (months)	71	2 (2-3)	2 (2-3)	0.450	Not Significant
Onset of Jaundice	52				
Within 24 hours after birth		3	5	0.648	Not Significant
1-2 days of Life		2	2	1.000	Not Significant

3-7 days of Life		6	12	0.247	Not Significant
1 to 4 weeks of life		8	14	0.323	Not Significant
More than 1 month of life		4	4	1.000	Not Significant
Birthweight (g)	17				
<2500 g		2	0	0.113	Not Significant
>2500 g		8	7		
Pale/Acholicstools	54				
Absent		9	3	0.469	Not Significant
Present		15	27		
Dark Urine	53				
Absent		7	5	0.057	Not Significant
Present		16	25		
Hepatomegaly	63				
Absent		15	10	0.231	Not Significant
Present		12	25		
Splenomegaly	62				
Absent		19	15	0.026	Significant
Present		5	11		
Ascites	51				
Absent		19	30	0.207	Not Significant
Present		2	2		

Table 1 shows that there are no significant differences on Patient Clinical Characteristic in terms of Gender, Onset of Jaundice, Birthweight, Pale/Acholic, Dark Urine, Hepatomegaly and Ascites. On the other hand, a significant difference between group 1 and 2 was observed with splenomegaly, a finding present in BIliary Atresia.

Table 2. Laboratory Test Results at the beginning of investigation, expressed as the median (interquartile range)

Variable	N	Intrahepatic	Extrahepatic	P	Interpretation
ALT IU/L	42	(n=19) 228 (176.5-542)	(n=23) 201 (163-658)	0.144	Not Significant
AST IU/L	29	(n=13) 331 (284-718)	(n-16) 270.5 (190.25-850)	0.448	Not Significant
ALP IU/L	30	(n=15) 598 (403.6-1412)	(n=15) 678 (512.28-1110)	0.412	Not Significant
GGT IU/L	8	(n=5)308 (296-679)	(n=3)882 (517.5-1054)	0.043	Significant
Albumin (g/dl)	17	(n=8) 29 (26.25-41)	(n=9) 32 (27-49)	0.606	Not Significant
Globulin (g/dl)	17	(n=8) 31 (26-38)	(n=9) 28 (23-53)	0.815	Not Significant
INR	55	(n=21) 1.05 (1.01-1.8)	(n=34) 1.01 (0.96-1.86)	0.052	Not Significant
TBIL (mg/dl)	44	(n=20) 205.81 (143.5-315)	(n=34) 186 (144-476.79)	0.075	Not Significant
DBIL (mg/dl)	44	(n=20) 159.335 (112.5-282)	(n=24) 148 (110.5-356.63)	0.944	Not Significant
Total Protein (g/dl)	16	(n=8) 64 (53.75-72)	(n=9) 61 (54-85)	0.956	Not Significant

Analysis of Table 2 shows that there is no significant differences between Group 1 and 2, except for GGT, being consistently elevated in Group 2.

Table 3. Histopathologic Features of Neonatal Cholestasis

Variable	Intrahepatic (n=32)	Extrahepatic (n=39)	P	Interpretation
Portal Ductal Proliferation				
Absent	31	5	0.000	Significant
Present	1	34		
Periportal Ductal Proliferation				
Absent	31	5	0.000	Significant
Present	1	34		

Bile Plugs				
Absent	29	11	0.029	Significant
Present	3	28		
Lobular Inflammation				
Absent to Mild	5	22	0.394	Not Significant
Moderate to Severe	27	17		
Giant Cell Transformation				
Absent to Mild	4	14	0.029	Significant
Moderate to Severe	28	25		
Fibrosis				
Absent	17	5	0.001	Significant
Portal	8	5	0.200	Not Significant
Periportal	5	7	0.205	Not Significant
Septal	1	5	0.000	Significant
Cirrhosis	1	17	0.000	Significant
Cholestasis				
Portal				
Absent	21	13	0.001	Significant
Present	11	26		
Neoductules				
Absent	28	13	0.000	Significant
Present	4	26		
Canalicular				
Absent	4	2	0.153	Not Significant
Present	28	37		
Intrahepatic				
Absent	1	2	0.065	Not Significant
Present	31	37		

Portal-Portal Bridges				
Absent	25	8	0.047	Significant
Present	7	31		
Portal Inflamation				
Absent	0	0	0.239	Not Significant
Present	32	39		
Extramedullary Hematopoiesis				
Absent	17	30	0.010	Significant
Present	15	9		
Ballooning Degeneration				
Absent	0	0	0.389	Not Significant
Present	32	39		
Necrosis				
Absent	20	20	0.477	Not Significant
Present/Focal	12	19		

Table 3 depicts the histopathologic parameters considered in adjudicating neonatal cholestasis. There was no significant difference between two groups on these parameters: Lobular Inflammation, Portal and Peri-portal fibrosis, Canalicular and Intrahepatic Cholestasis, Portal Inflammation, Ballooning Degeneration and Necrosis. On the other hand, significant differences between Group 1 and 2 were observed on these parameters: Presence of bile plugs, Portal Ductal Proliferation, Periportal Ductal Proliferation, Giant Cell Transformation, Absence of fibrosis, Septal FIbrosis and Cirrhosis, Portal and Neoductular Cholestasis, Portal-Portal Bridges and extramedullary hematopoiesis.

For ultrasound and HIDA scan findings, only the presence of a normal gall bladder for intrahepatic cholestasis achieved statistical significance.

Overall, 81 patients underwent liver biopsy with a clinical diagnosis of Neonatal Cholestasis. Of these, only 71 were included in the study, because 5 of the cases have no slides and block to retrieve for review (these were probably borrowed and were not returned), while the remaining 5 have absent or scanty portal tracts to be adequately evaluated.

Seventy – one cases included in the study were finally grouped into two. Thirty-two cases (45%) were classified as Group 1 or intrahepatic group, and 39 cases (55%) as Group 2 or extrahepatic group. The etiologies of hepatitis for

group 1 are Cytomegalovirus Hepatitis(6 cases, 16%), Herpes Simplex II Hepatitis(1 case, 3%), and Unidentified or Possibly Idiopathic (25 cases, 81%). All viral-induced Hepatitis for group 1 cases was diagnosed based on positivity on TORCH IgM. For the etiologies of Cholestasis for Group 2, these are Biliary Atresia (33 cases, 85%), Inspissated Bile/Bile Sludge (4 cases,10%), Choledochal Cyst (1 case,2.5%) and Common Bile Duct Stone (1 case, 2.5%).

Final Diagnosis was based on Intraoperative Cholangiography findings, Hepatobiliary exploration findings, and Histopathology findings. In our study, 12 biliary atresia cases and all Inspissated Bile/Bile Sludge cases were confirmed by Intraoperative Cholangiography.The case of Choledochal Cyst and Common Bile duct obstruction were confirmed by surgical exploration. The 21 cases of Biliary atresia were confirmed by Histopathology findings. Six cases of biliary atresia are Cytomegalovirus-associated as confirmed by Urine CMV Culture(2 cases) and TORCH IgM(4 cases). One patient included in Group 2 is a case of Trisomy 21(Down Syndrome). Three postmortem liver biopsy were included in the study, two belonging to Group 2 with final diagnosis of Extrahepatic Biliary Atresia with cirrhosis and Extrahepatic Biliary Atresia with Cirrhosis with Cytomegalovirus Hepatitis(IgM Positive) and one belonging to Group 1 with final diagnosis of Hepatitis with Cirrhosis.

In this study, we evaluated 25 cases of core biopsy and 7 cases of wedge biopsy for group 1. For Group 2, there were 21 cases of core biopsy, 12 cases of wedge biopsy, and 6 cases with both core and wedge biopsy. No difference was noted on cases having both core and wedge biopsy on evaluation, hence the histologic findings were considered as one.

Review of the histopathology reports showed that 3 of the 32 cases of group 1 would need additional Cytokeratin IHC for confirmation of the diagnosis, and 16 of 39 cases of group 2 would need Cytokeratin for confirmation of diagnosis. IHC with Cytokeratin were all done on these cases and were evaluated with the H&E slides. Comparing the provisional diagnosis without Cytokeratin with the final diagnosis with Cytokeratin, only 2 of 19 cases in which Cytokeratin was requested resulted in revision of diagnosis, and these cases were initially diagnosed in favor of Hepatitis but after doing Cytokeratin the final diagnosis showed Bile Duct proliferation consistent with a diagnosis of Biliary Atresia. For these two cases, the proliferating bile ducts were obscured by severe portal and periportal inflammation.

Included in the study are 41 males (58%) and 30 females (42%) patients. There were 17 males and 15 females comprising 53 % and 47% of group 1 respectively, and 24 males and 14 females comprising 61% and 39% of group 2 respectively. No significant differences were noted as to sex distribution in both groups. We also found no significant differences on the age of patient at time of biopsy. All presented with jaundice and most presented within 3 to 7 days of life and one to four weeks of life. Almost all patients have birthweight >2500 grams. While most patients presented with pale/acholic stools (78%), choluria (77%), and hepatomegaly (59%), no significant differences were noted on these 3 parameters. Few patients presented with splenomegaly(26%) and ascites(7%). Of these two parameters, a significant difference is noted among

patients presenting with splenomegaly which is more likely found on patients with Biliary Atresia. No significant difference was obtained on patients presenting with ascites on both groups.

On all the Laboratory parameters evaluated, using the median of all the laboratory results per parameter, Only GGT showed significant differences between the two groups.(Table 2)

The histopathologic features for each group are summarized in Table 3. The characteristic features belonging to Group 1 include absence of fibrosis which is noted on 53% of patients in Group 1 but only 13% of patients in Group 2, and presence of Extramedullary Hematopoiesis which is seen in 47% of patients belonging to group 1 but only in 23% of patients belonging to Group 2. Also quite prominent is the degree of giant cell transformation in which moderate to severe giant cell transformation is seen in 88% of group 1 and only in 64% of patients in group 2 while absent to mild giant cell transformation is noted only in 12 % of group 1 and 36% of group 2. Thus, a significant difference is noted on both groups with regards to the degree of giant cell transformation.

The characteristic histopathology features of patients belonging in group 2 includes: portal and periportal ductal proliferation which are seen in 87% of patients in group 2 and only in 3% of patients in group 1; presence of bile plugs which are seen in 72% of patients belonging to group 2 and only in 9% of patient in Group 1; presence of septal fibrosis seen in 13% of patients in group 2 and only in 3% of patients in group 1; presence of cirrhosis which is seen in 44% of patients in group 2 and only in 3% of patient in group 1, presence of Cholestasis in the portal tract seen in 67% of patients in group 2 and only in 33% of patients in group 1; presence of cholestasis in the neoductules seen in 67% of patients in group 2 and only in 13% of patients in group 1; and, lastly, presence of portal-portal bridges which is seen in 79% of patients in Group 2 and only in 29% of patient in group 1. Other histopathologic variables evaluated but were found not significant are Degree of lobular Inflammation, Portal and Periportal Fibrosis, Canalicular and Intrahepatic Cholestasis, portal inflammation , ballooning degeneration, and necrosis.

Only 28 Ultrasound results were present on the patient's charts, 15 for group 1 and 13 for group 2. Results show that the finding of a normal gallbladder predominated group 1 while finding of atretic/small gallbladder is more common in Group 2. Findings of an absent or nonvisualized gallbladder and contracted gallbladder do not significantly differ on both groups.

Intraoperative Cholangiography (IOC) is the gold standard in the diagnosis of biliary atresia. Of the 23 cases with IOC, 12 had findings of Biliary Atresia, 4 had Inspissated Bile/ Bile Sludge, and 7 had diagnosis of Cholestasis with negative exploration. Eleven cases of Biliary Atresia had similar IOC findings with histopathology results, however 2 cases have differing diagnosis. Of these two cases, one case has an IOC finding of Biliary Atresia while the Liver Biopsy finding showed Neonatal Hepatitis and for the second case IOC showed negative exploration while the liver biopsy showed findings consistent with Biliary Atresia. On review of both cases, we found consistent findings with the histopathology report. These could happen for several reasons as follows: ductular reaction may occur in patients with Neonatal Hepatitis which may not seem conspicuous in a core biopsy specimen, in early cases of biliary atresia

bile duct proliferation is not prominent, thus a repeat biopsy is recommended, and some diseases may mimic biliary atresia histologically like Neonatal Sclerosing Cholangitis, CMV Hepatitis, alpha-1 antitrypsin deficiency and Total Parenteral nutrition[10]. Four cases have IOC findings of Inspissated bile/Bile Sludge but a diagnosis of Neonatal Hepatitis on Histopathology report. Histopathologic changes in inspissated bile is nonspecific and can mimic changes consistent with Neonatal Hepatitis thus it cannot be diagnose by Liver Biopsy. Clinical Correlation is warranted in such cases. Comparing the Liver Biopsy findings among cases with IOC findings of Biliary Atresia, Liver Biopsy yielded sensitivity of 92%, specificity of 86%, positive predictive value of 92%, negative predictive value of 86%, and accuracy of 89%.

DISCUSSION

The focus of initial approach in this study is to differentiate between Intrahepatic Cause and Extrahepatic Cause, since an Extrahepatic cause of obstruction would warrant surgical intervention while only medical intervention is needed for the Intrahepatic group.

Idiopathic Neonatal Hepatitis represents 15% of patients presenting with Neonatal cholestasis[9]. It is diagnosed after a thorough history, physical examination and laboratory evaluation fail to identify an underlying cause of Neonatal Hepatitis[5]. Cytomegalovirus Hepatitis is second to idiopathic neonatal Hepatitis, representing 3-5% of Neonatal Hepatitis.

Among the extrahepatic causes of cholestasis, Biliary Atresia represents 25 to 30% of patients with neonatal cholestasis[9]. Biliary atresia is the most important cause of severe neonatal liver disease and the major indication for liver transplant, since Intrahepatic Bile Duct Damage continues to occur which leads to loss of intrahepatic bile ducts and recurrent Cholestasis due to Bile Duct Paucity even after a successful Kasai procedure [10].

Diagnosis of Cholestasis is a matter of urgency since biliary atresia is a common cause of Cholestasis. The goal of management is to complete the diagnostic evaluation, or at least exclude biliary atresia by 45 to 60 days of life [7]. The prognosis for a successful Kasai or portoenterostomy procedure depends primarily on operation before 60 days of age and absence of cholangitis[9]. CMV infection in combination with a genetic predisposition may play a role in the development of perinatal pattern of biliary atresia. In our studywe found 6 out of 33 cases of Biliary Atresia positive for CMV as confirmed by serology and Urine CMV. One of the six cases is a postmortem liver biopsy which may entail worse prognosis as compared to biliary atresia alone. This is similar to the study done by Tarr and colleagues, wherein 5 of 23 patients with Biliary Atresia had evidence of CMV hepatitis based on serology, culture and histopathological evidence[9].

In our study idiopathic neonatal hepatitis and biliary atresia accounted for 46% and 37% respectively of Neonatal Cholestasis among patients who underwent Liver Biopsy. This is almost similar to studies done in Brazil [2] and Malaysia [7] in which both diseases accounted for 54% and 67% of cases of Neonatal Cholestasis respectively.

Cytomegalovirus Hepatitis is the most common perinatal infection seen in 40 to 50% of infants delivered to mothers with primary CMV. Six out of 32(16%) cases in group 1 are positive for CMV IgM. Liver diseases range

from mild portal inflammation to severe giant cell hepatitis to cirrhosis. Another congenital infection seen in one case in group 1 (3%) is Herpes Simplex Virus type 2, a virus responsible for almost 80% of perinatal infections. Disseminated infection which results in fulminant hepatic failure occur in approximately 20% of newborns. Hepatocyte necrosis devoid of inflammatory cells and viral inclusions are the microscopic features of this entity[9].

Inspissated bile syndrome(IBS) is obstructive jaundice caused by intraluminal bile plugs, sludge or gallstones and is uncommon in infancy. In a study by Redkar and colleagues, possible predisposing factors include gastrointestinal pathology, prematurity, total parenteral nutrition, sepsis, and other miscellaneous causes like IUGR, birth asphyxia, and hemolysis. Biliary ultrasound was the most useful primary investigation and diagnostic tool. Inspissated bile and dilated biliary tree are common findings. Underlying structural anomalies of the bile ducts identified in the same study by Redkar were choledochal malformation, stricture, abnormal ductal anatomy, and a long common channel. Thus, Inspissated bile syndrome is a cause of surgical jaundice in this age group and is of heterogenous etiology. Majority will require intervention, either radiological or surgical, but with excellent long-term outcome[8].

One cause of neonatal cholestasis is Choledochal Cyst, representing 2.5% of the extrahepatic causes of cholestasis in the study. Choledochal cyst is a segmental dilatation of the biliary ductal system. It is usually diagnosed by ultrasound, however in neonates it is important to distinguish this lesion from biliary atresia.

Another cause of cholestasis in our study is obstruction by common bile duct stones, and it represents 2.5% of extrahepatic causes of cholestasis. It is very rare in neonates and infants and occurs only in approximately 0.13% to 0.22% in ultrasound-based studies[9]. It can be associated with prematurity, inspissated bile/stasis, correctable biliary atresia, and choledochal cyst.

Among the clinical parameters evaluated, only the finding of splenomegaly would favor a diagnosis of an extrahepatic cause of cholestasis. This could be very well correlated with the presence of cirrhosis predominantly seen in the same group causing increase in portal pressure secondary to blockage of blood flow in the portal vein and obstruction of bile flow. Splenomegaly represents a late sequelae of a disease particularly in Biliary Atresia. This would indicate either the severity of obstruction, late presentation of the disease and/or possible delay in arriving at the diagnosis.

Laboratory parameters evaluated in this study include: Bilirubin, which is a test for metabolic function of the liver; albumin and INR, which are tests for the synthetic function of the liver; AST and ALT, which are tests for liver injury; and ALP and gamma glutamyl transferase (GGT), which are tests for canalicular injury. In this study, high GGT levels would favor a diagnosis of Biliary Atresia. GGT has 78-86% sensitivity and 67-100% specificity for obstruction. GGT has been used in the past to distinguish biliary atresia from neonatal hepatitis; however a wide variability in levels makes interpretation difficult[5]. Normal values of GGT varies with age, gender and diagnostic methods. It is recommended that GGT results be expressed as the number of times the upper limit of normaland a cut-off value

be set to differentiate Biliary Atresia from other causes of Cholestasis[2].

Giant Cell transformation of hepatocytes is seen in 15% of Extrahepatic Biliary Atresia and may occasionally be prominent [10]. In this study, a less degree of giant cell transformation is noted in favor of Biliary Atresiaand a marked degree of Giant Cell transformation favors Neonatal Hepatitis. Our findings are Similar to the study by Santos and Alagille which include extramedullary hematopoiesis among the variables that favor an intrahepatic cause of cholestasis.

In our study, portal and periportal Ductal Proliferation were findings indicating an extrahepatic cause of cholestasis as also demonstrated by Brough and Bernstein in 1974 and Santos and colleagues in 1998. There are two conflicting studies on the findings of cholestasis in Neoductules; Brough and Bernstein considered it as a non specific finding, while Shiraki and colleagues considered it as the most specific discriminatory element. Similar to the findings of Santos, which showed that Portal Cholestasis and Cholestasis in Neoductules are directly correlated and are discriminatory variables indicating an extrahepatic cause of cholestasis. Portal-Portal bridges or fibrosing piecemeal necrosis is a reversible phenomenon; in our study, similar to that of Zerbini and Santos, we found this variable as indicative of an extrahepatic cause of cholestasis[3]. Similar to the study by Lee and colleagues, the occurrence of septal fibrosis and cirrhosis were significantly greater in patients with Extrahepatic Cholestasis[1].

Ultrasonography is use to identify anatomic abnormalities such as choledochal cyst. Findings of a small or absent gallbladder may suggest extrahepatic biliary atresia but with low sensitivity of 73%. Combination of three gallbladder features (gallbladder ghost triad) namelylength less than 19 mm,irregular wall, and an indistinct mucosal lining for experienced operators yielded >90% sensitivities and specificities[11].No data regarding these findings were noted on the results evaluated. In our study, only the finding of a normal gallbladder is significant in differentiating intrahepatic from extrahepatic cholestasis. Presence of triangular cord sign on ultrasound has been considered a significant diagnostic feature of biliary atresia with high sensitivity and specificity[7]. Triangular cord sign is a focal area of increased echogenicity anterior to the bifurcation of the portal vein representing the fibrotic remnant of the extrahepatic biliary tree[11]. However, these appears to be operator dependent since in our study none of the ultrasound results of patients with Biliary Atresia have findings of triangular cord sign. We emphasized that multiple ultrasound parameters should be analyzed to have an accurate diagnosis of biliary atresia.

CONCLUSION AND RECOMMENDATION

Biliary Atresia and Idiopathic Neonatal Hepatitis represent the majority of the causes of Neonatal Cholestasis comprising 46% and 37% respectively.

Findings of splenomegaly and markedly elevated GGT favor an extrahepatic cause of cholestasis while ultrasound finding of a normal gallbladder favors an intrahepatic cause of cholestasis.

Liver biopsy is essential in making an accurate diagnosis with a high Sensitivity (92%), Specificity (86%) and Accuracy (89%). We recommend percutaneous Liver Biopsy to all patients with cholestasis

particularly in cases with diagnostic uncertainty and even in CMV positive cases.

Histopathology findings of Portal and Periportal Ductal proliferation, bile plugs, lesser degree of giant cell transformation, septal fibrosis and cirrhosis, portal and neoductular cholestasis, and Portal-Portal bridges indicate an extrahepatic cause of cholestasis. A marked degree of giant cell transformation and presence of extramedullary hematopoiesis favor an intrahepatic cause of cholestasis. These Histopathologic parameters would help the pathologist in making a definitive diagnosis. We recommend the use of Cytokeratin IHC on all tissues showing obliteration of portal tracts due to fibrosis or inflammation for proper evaluation.

The biggest drawback of the study is the incomplete clinical and laboratory data for evaluation. This is due to incomplete documentation of relevant history and physical examination findings, the failure of incorporating the results of different laboratory tests and ancillary procedures in the charts, and the non-performance of some critical laboratory tests and procedures. Significantly limiting the number of cases and parameters for evaluation in this study are unrecovered slides, paraffin blocks, and missing charts of patients. A prospective study is recommended since it would better document and archive the data needed. It is likewise beyond the scope of this study to determine and follow-up the clinical outcome of the patients. Determining the patients' outcome would support the diagnosis and determine the prognosis and disease process.

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