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Original Research Paper

Pathology

PATTERNS OF INTRAHEPATIC CHOLESTATSIS ON LIVER BIOPSY

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ABSTRACT Histologically, cholestasis is presence of visible bile in tissue sections. Normally liver biopsy does not show presence of bile, if present it is always pathological. Cholestasis can be either intrahepatic or extrahepatic. Extrahepatic causes are mostly diagnosed with radiological investigations like USG, CT and MRI. Liver biopsy is indicated in intrahepatic causes of cholestasis. Intrahepatic cholestasis on liver biopsy shows four patterns namely acute cholestasis with large duct obstruction, bland cholestasis, intrahepatic bile duct loss or ductopenia and cholestasis associated with hepatitis. These four patterns are derived based on the histological changes seen in liver parenchyma and portal tract.

Summary: We have studied different patterns of intrahepatic cholestasis and shortlisted a number of probable etiologies. In addition to the patterns of intrahepatic cholestasis, we also studied other histologic features seen in the liver parenchyma and portal tract and correlated our findings with clinical history, laboratory investigations and radiological investigations to reach a final diagnosis.

KEYWORDS : Cholestasis, Intrahepatic, Patterns

INTRODUCTION

Cholestasis is defined as defect in bile secretary mechanism leading to accumulation of substances in blood, which are normally excreted in bile.[1]

Histologically, cholestasis is presence of visible bile in tissue sections. Normally liver biopsy does not show presence of bile, if present it is always pathological.

Cholestasis can be either intrahepatic or extrahepatic.

Extrahepatic causes are mostly diagnosed with radiological investigations like USG, CT and MRI.[1]

Liver biopsy is indicated in intrahepatic cholestasis.[2]

The systemic approach to reach the diagnosis in a patient with suspected cholestasis should be in the following manner:

1. Firstly, thorough history followed by physical examination
2. Laboratory investigations
3. Abdominal imaging – USG, CT, MRI
4. Serology studies and
5. Lastly, liver biopsy

AIMS AND OBJECTIVES

a. To evaluate patterns of intra-hepatic cholestasis on liver biopsy in adults.
b. To study probable etiology of intrahepatic cholestasis.
c. To determine the association between patterns of intrahepatic cholestasis and etiology.

MATERIALS AND METHODS

Retrospective observational study over a period of 5 years - January 2011 to December 2015.

Institutional ethics committee clearance was obtained.
Sample size – 70

Inclusion criteria:

- All adult liver biopsies showing intrahepatic cholestasis in which complete clinical information was available.

Exclusion criteria

- Extra hepatic cholestasis
- All patients with malignancy showing cholestasis
- Patient with inadequate biopsy specimen
- Paediatric biopsy specimens
- Biopsy with cholestasis but without any clinical information

Already processed & stained slides of liver biopsy referred to principal investigator showing intrahepatic cholestasis were reviewed.

Review was done by the principal investigator in blinded fashion (without knowing the clinical findings, laboratory and radiological investigations) and thereafter viewed by co-investigator.

Liver biopsy in case of cholestatic disease shows many changes in the portal tract as well as in the liver parenchyma.

Changes in the portal tracts like portal tract oedema, duct loss, ductopenia, portal tract proliferation, neutrophils around proliferating ducts, ductular epithelial changes and periductal fibrosis were noted.

Changes in the parenchyma namely feathery degeneration, ballooning degeneration, ground glass hepatocytes, Mallory bodies, steatosis, fibrosis and cirrhosis were looked for.

Site of cholestasis that is whether it is at the level of hepatocytes (figure1), bile canaliculi (figure2) and/or bile ductules (figure 3) was mentioned.[2]

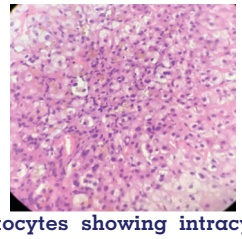

Figure 1:Hepatocytes showing intracytoplasmic choles tasis (H&E 400X)

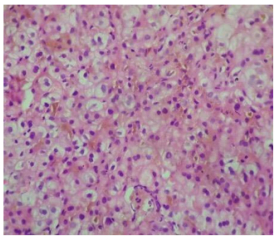

Figure 2: Canalicular cholestasis (H&E 400X)

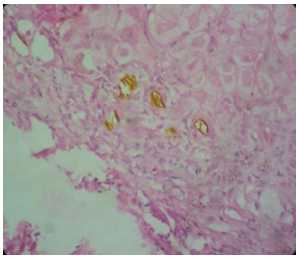

Figure 3: Cholestasis in bile ductules (H&E 400X)

Above findings were formulated as present or absent and based on the findings, liver biopsies were divided into four patterns[3]

1. *Acute cholestasis in large duct obstruction*
2. *Bland cholestasis*
3. *Intrahepatic bile duct loss/ductopenia*
4. *Cholestasis associated with hepatitis (Viral hepatitis, autoimmune hepatitis, drugs)*

RESULTS AND DISCUSSION

Acute cholestasis with large duct obstruction included 2 cases of PSC (Primary Sclerosing Cholangitis) and sepsis each. These 2 cases of PSC showed prominent ductular reaction, moderate to severe degree of intracytoplasmic and canalicular cholestasis, with one case showing periductal fibrosis and other case showing ductular epithelial changes, probable diagnosis based on these findings were PBC (Primary Biliary Cirrhosis), PSC and sepsis. On unveiling of clinical examination, laboratory investigations and radiological findings these cases revealed raised serum ALP (Alkaline Phosphatase) and GGT (Gamma Glutamyltr ansferase) levels with p-ANCA positive in both case, no IHBRD (Intrahepatic Biliary Radicle Dilatation) was seen in both the cases so final diagnosis given was PSC.

The other two cases also showed ductular reaction with significant neutrophilic inflammatory infiltrate in portal tracts forming abscess in portal tracts with severe degree of bile ductular cholestasis. The probable diagnosis given on histological examination were PBC, PSC, sepsis and overlap syndrome but clinical and radiological correlation revealed a raised AST/ALT, GGT, ALP, Bilirubin with hepatolithiasis which led us to the final diagnosis of sepsis with features of large duct obstruction.[5]

Bland cholestasis pattern showed normal portal tracts with mild to moderate inflammation, few parenchymal changes like steatosis, copper deposition and moderate to severe degree of canalicular and cytoplasmic cholestasis.[6]

The differential diagnosis given based on histologic picture were BRIC (Benign Recurrent Intrahepatic Cholestasis), DILI (Drug Induced Liver Injury), Sepsis and Wilson's disease. The final diagnosis of BRIC was given in patients who had recurrent episodes of jaundice, complain of severe pruritus, positive history of similar complains in family members, raised ALP but normal GGT levels with no history of intake of drugs or pregnancy.

Diagnosis of DILI under bland cholestasis was given in cases which had above mentioned histologic features with positive history of intake of anabolic steroids and OC pills (drugs showing bland cholestasis).

Diagnosis of Wilsons disease under above mentioned category was given in patients which in addition to above findings showed variable degree of cirrhosis, piecemeal and panlobular necrosis with positive stains for copper (orcein).[8]

Pattern showing duct damage and duct loss included 8 cases of PBC, 6 cases of PSC, 2 cases of DILI, 4 cases of overlap syndrome and 1 granulomatous hepatitis.

Cases of PBC showed ductopenia, duct damage with few showing prominent ductular reaction, significant lymphoid cell infiltrate in portal tract, granulomas in portal tracts and parenchyma showing bridging necrosis, fibrosis to cirrhosis, feathery degeneration of hepatocytes (figure 4), moderate to severe intracytoplasmic to canalicular cholestasis.[9]

The probable diagnosis given based on these histologic features were chronic hepatitis C, AIH (Autoimmune Hepatitis), PBC, PSC and granulomatous hepatitis. On unveiling of other investigations, all these cases had complains of jaundice, pruritus, raised ALP, GGT, serum bilirubin levels, mild to moderate rise in SGOT/SGPT levels, positive AMA titres , radiology showed cirrhosis and altered liver parenchyma, clinical impression was variable ranging from acute on chronic liver failure, AIH, chronic liver disease to PBC. Based on correlation of histology, clinical findings and investigations these cases were concluded as PBC.

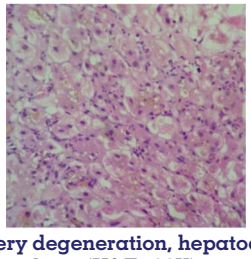

Figure 4: Feathery degeneration, hepatocytes with rarefied and reticular cytoplasm (H&E 400X)

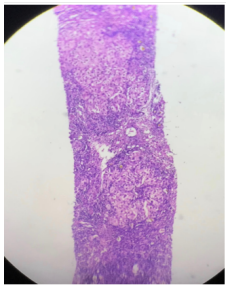

Figure 5: Biliary cirrhosis, Jigsaw pattern (H&E 100X)

Of the total 8 cases of PSC, 6 cases showed this pattern of cholestasis. These cases showed variable presence of ductopenia, classic "onion skin fibrosis", biliary pattern of cirrhosis (figure 5), feathery degeneration of hepatocytes and moderate to severe degree of intracytoplasmic and canalicular cholestasis. On viewing the detailed work up of the patient, one case was a known case of PSC, 2 cases had history of inflammatory bowel disease, complains of jaundice, pruritus with increase in SGOT/SGPT levels, raised ALP, GGT, serum bilirubin and positive titres for p-ANCA, AMA, ASMA , on cholangiography all cases had IHBRD, and clinical impression was PSC in 5 of the cases.

Final HP Diagnosis		Patterns of cholestatsis											
		Acute cholestasis in large duct obstruction		P value	Bland cholestasis		P value	Intrahepatic bile duct damage or loss		P value	Cholestasis associated with hepatitis		P value
		Present	Absent		Present	Absent		Present	Absent		Present	Absent	
Acute Hepatitis	Count	0	5	–	0	5	–	0	5	–	5	0	**0.008 S**
	%	0.0%	100.0%		0.0%	100.0%		0.0%	100.0%		100.0%	0.0%	
AIH	Count	0	3	–	0	3	–	0	3	–	3	0	0.059 NS
	%	0.0%	100.0%		0.0%	100.0%		0.0%	100.0%		100.0%	0.0%	
BRIC	Count	0	6	–	6	0	**0.0004 S**	0	6	–	0	6	–
	%	0.0%	100.0%		100.0%	0.0%		0.0%	100.0%		0.0%	100.0%	
Chronic hepatitis B	Count	0	10	–	0	10	–	0	10	–	10	0	**0.0001 S**
	%	0.0%	100.0%		0.0%	100.0%		0.0%	100.0%		100.0%	0.0%	
Chronic hepatitis C	Count	0	6	–	0	6	–	0	6	–	6	0	**0.002**
	%	0.0%	100.0%		0.0%	100.0%		0.0%	100.0%		100.0%	0.0%	
DILI	Count	0	9	–	5	4	0.032	2	7	0.714 NS	2	7	0.298 NS
	%	0.0%	100.0%		55.6%	44.4%		22.2%	77.8%		22.2%	77.8%	
Granulomatous hepatitis	Count	0	1	–	0	1	–	1	0	–	0	1	–
	%	0.0%	100.0%		0.0%	100.0%		100.0%	0.0%		0.0%	100.0%	
Inconclusive	Count	0	2	–	2	0	–	0	2	–	0	2	–
	%	0.0%	100.0%		100.0%	0.0%		0.0%	100.0%		0.0%	100.0%	
Overlap syndrome	Count	0	6	–	0	6	–	4	2	0.061 NS	2	4	1.00 NS
	%	0.0%	100.0%		0.0%	100.0%		66.7%	33.3%		33.3%	66.7%	
PBC	Count	0	8	–	0	8	–	8	0	**0.0001 SS**	0	8	–
	%	0.0%	100.0%		0.0%	100.0%		100.0%	0.0%		0.0%	100.0%	
PSC	Count	2	6	0.061 NS	0	8	–	6	2	**0.007 S**	0	8	–
	%	25.0%	75.0%		0.0%	100.0%		75.0%	25.0%		0.0%	100.0%	
Sepsis	Count	2	1	–	1	2	–	0	3	–	0	3	–
	%	66.7%	33.3%		33.3%	66.7%		0.0%	100.0%		0.0%	100.0%	
Wilsons disease	Count	0	3	–	3	0	–	0	3	–	0	3	–
	%	0.0%	100.0%		100.0%	0.0%		0.0%	100.0%		0.0%	100.0%	

The 2 cases of DILI included under this pattern showed ductopenia, ductular epithelial changes, 1 case showed significant (+++) eosinophilic inflammatory infiltrate in portal tract, with parenchyma showing bridging necrosis, moderate to severe intracytoplasmic and canalicular cholestasis. The probable diagnosis based on the above findings were DILI and PBC. On exposure to detailed history and investigations, both these cases had history of long term use of hepatotoxic medication, one case had history of series of dental extraction and on going amoxicillin-clavulanic acid treatment course for the same while the other case had history of carbamazepine intake for psychosis. Laboratory investigations showed deranged SGOT/SGPT, raised ALP, GGT, serum bilirubin with ultrasonography of abdomen showing no significant abnormality in one case and altered liver parenchyma in other case. Our final diagnosis based on correlating the histology findings with history, clinical presentation, laboratory investigations, radiological investigations and clinical impresssion were DILI induced ductopenia.

One case of granulomatous hepatitis showed portal tracts filled with epithelioid cell granulomas, Langhan's giant cells in portal tract and parenchyma with marked ductular damage and mild canalicular cholestasis ,probable diagnosis given was granulomatous hepatitis and PBC, on further evaluation this case was a diagnosed case of tuberculosis, had near normal SGOT/SGPT levels, raised ALP, GGT, serum bilirubin levels, radiology was suggestive of infilrative disease of liver, clinical impression was granulomatous hepatitis. Finally, considering all findings this case was put under granulomatous hepatitis with pattern of cholestasis showing duct damage and loss.

4 cases of overlap syndrome showed presence of ductular reaction, ductular epithelial changes, significant lymphoplasmacytic inflammatory infiltrate in portal tracts, periductal fibrosis, duct loss, granulomas in portal tract, piecemeal necrosis, bridging necrosis, cirrhosis, fibrosis, feathery degeneration of hepatocytes, moderate to severe degree of intra-cytoplasmic and canalicular cholestasis. Based on the histologic findings probable diagnosis given were chronic hepatitis B, PBC, PSC, overlap syndrome.on unveiling of entire work up of these cases it revealed that 2 cases having positive titres of ANA, p-ANCA, ASMA with IHBRD on cholangiography in addition to raised ALP, GGT and serum bilirubin levels and history of UC these were labelled as AIH + PSC. Rest of the 2 cases were labelled as AIH+PBC had AMA, ANA titres with raised IgG levels and picture of cirrhotic liver on radiology.

Pattern of cholestasis associated with hepatitis Majority of cases were grouped under this pattern of cholestasis. It included cases of chronic hepatitis B, chronic hepatitis C, acute hepatitis, AIH, DILI, overlap syndrome.

6 cases of chronic hepatitis C on histology showed lymphoid aggregates in the portal tracts few showing germinal centre, hepatitis rosettes, periportal steatosis, portal to portal bridging necrosis, piecemeal necrosis, fibrosis and cirrhosis, one case showed giant cell transformation, moderate to severe cytoplasm and canalicular cholestasis.[10] Probable diagnosis based of these histologic findings were chronic hepatitis C, chronic hepatitis B, AIH and PBC. On viewing the entire work up, these cases had HCV RNA positive, one was a known case of hepatitis C, there was positive history of blood transfusion in past in 2 cases, one case was retrovirus positive, laboratory investigations of these patients revealed normal to deranged SGOT/SGPT levels, normal to slightly raised ALP and GGT levels, raised serum bilirubin levels.On radiology, impression was cirrhosis and liver parenchymal disease.

Chronic hepatitis B was the final diagnosis in 10 cases, histology of these cases revealed ballooning degeneration of hepatocytes, ground glass hepatocytes, central vein to portal tract bridging necrosis, glycogenated nuclei of hepatocytes, moderate to severe degree of intracytoplasmic and canalicular cholestasis. Probable diagnosis given based on the afore mentioned findings were AIH, chronic hepatitis C and chronic hepatitis B. On unveiling of complete details, all cases presented with jaundice and variably present other symptoms like abdominal pain, abdominal distension, pruritus, deranged SGOT/SGPT levels, normal to slightly raised ALP, GGT and elevated serum bilirubin levels. All patients were positive for hepatitis B surface antigen. Cirrhosis was evident on radiology. Clinical impression received in all cases of hepatitis B were chronic liver disease under evaluation.

2 cases of overlap syndrome were cases which had features of both AIH and PBC, but features of AIH more predominant than features of PBC, on histology. They showed presence of ductular reaction, duct epithelial changes, significant lymphoplasmacytic infiltrate in portal tracts, hepatic pseudo rosettes (figure 6), piecemeal necrosis, bridging necrosis, cirrhosis and moderate degree of intracytoplasmic and canalicular cholestasis. Probable etiology given by us included overlap syndrome, AIH and Chronic hepatitis B. Investigations of these patients revealed elevated ANA titres, raised IgG levels, raised SGOT/SGPT, elevated ALP, GGT and serum bilirubin levels, jaundice, abdominal pain and distension as presenting complaints. Cirrhosis was found on radiology and clinical impression was of AIH.

5 cases of acute hepatitis showed extensive lobular inflammation, neocholangioles, confluent necrosis, feathery degeneration of hepatocytes, severe degree of intracytoplasmic and intracanalicular cholestasis. Probable diagnosis which were thought of were acute hepatitis and DILI. Investigations revealed elevated levels (>1000 IU/L) SGOT/SGPT, serum bilirubin and mild to moderate increase in ALP and GGT levels. 4 cases were positive for HAV with one case showing HEV positivity, clinical impression was intrahepatic cholestatic jaundice with radiology showing no significant abnormality.

Cases of AIH showed significant lymphoplasmacytic inflammatory infiltrate in portal tracts, piecemeal necrosis, pseudo rosettes, cirrhosis, giant cell transformation of hepatocytes (figure 7) and moderate intracytoplasmic and canalicular cholestasis. Probable diagnosis were autoimmune hepatitis and DILI. on investigations all cases showed strongly positive ANA titres with one case showing anti- LKM antibody positivity and elevated IgG titres, SGOT/SGPT , serum bilirubin, ALP/GGT showed raised levels. Clinical impression in all three cases were AIH whereas radiology showed no significant findings.

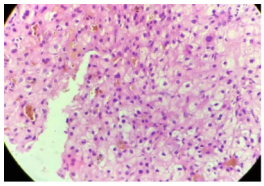

Figure 6: Hepatic pseudo-rosettes (H&E 400X)

Table 1 : Showing statistical significance between a group of diagnosis and the pattern they repeatedly showed on liver biopsy.

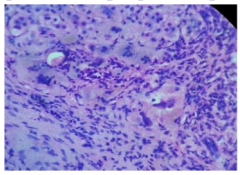

Figure 7: Post infantile giant cell transformation of hepatocytes (H&E 400X)

Statistically significant association was noted between BRIC and the pattern of Bland cholestasis (p value = 0.0004) (Table 1). In this pattern there was intrahepatocytic and canalicular cholestasis without significant portal tract pathology. (2)

Statistically significant association was noted between PBC (p value = 0.0001) (Table 1) and PSC (p value = 0.007) with the pattern of cholestasis associated with bile duct loss/ductopenia. In this pattern there was bile duct epithelial damage or duct loss.

However statistically significant relation could not be established between various etiologies like DILI, AIH, sepsis, overlap syndrome and granulomatous hepatitis with different patterns of cholestasis considered in our study. This was due to either the small sample size or a single etiology presenting with different patterns of cholestasis (for example, cases of DILI).

The pattern of cholestasis associated with hepatitis was found to be statistically significant (SS) in all 5 cases of acute hepatitis and all cases of chronic hepatitis B and C as all of them showed lobular disarray, ballooning and apoptosis of hepatocytes with portal tract inflammation which is a feature of this pattern.

The pattern of bland cholestasis was found to be statistically significant in all 6 cases of BRIC as all of them showed centrilobular cholestasis within hepatocytes and/or bile canaliculi without significant portal tract pathology.

The pattern of cholestasis associated with intrahepatic bile duct disease (duct damage/duct loss/ductopenia) showed SS in all cases of PBC and majority cases of PSC where both inflammatory and fibrosing type of duct damage was seen in respective etiologies.

CONCLUSION

Our study aimed at analyzing patterns of intra-hepatic cholestasis on liver biopsy to help us narrow down the list of probable etiologies and to reach a final diagnosis after complete knowledge of clinical history, laboratory investigations and radiological imaging.

So we concluded from our study that analysis of different patterns of cholestasis helps us to short list the probable etiology/diagnosis however complete correlation with clinical, radiological and laboratory investigations is needed to reach the final diagnosis.

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