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Liver diseases in the school going child (4-15 years)

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ABSTRACT

Background: The aim of our study was to study the clinical profile, evaluate the findings in various liver and hepatobiliary diseases on liver biopsy in pediatric patients (age group 4-15years). Also, to correlate the histopathological findings with the possible clinical presentation in chronic liver disease, to assess the prognosis, outcome and effects of treatment of chronic liver disease proven by biopsy.

Materials and methods: In this 5 year study, all liver biopsies and selective autopsy cases of children between 4-15 years of age were included. Wilson's work up included tests like S.ceruloplasmin, 24 hour urinary copper, post penicillamine 24 hour urinary copper, Kayser Fleischer ring, dry weight of copper, MRI Brain and family screening. The liver biopsies were stained with hematoxylin and eosin (H &E) stain. Also reticulin stain was done in all cases.

Results: In 57 cases, majority were in the 4-7 year age group (49.1%). The most common presenting feature was jaundice (64.9%). The most common histopathological diagnosis included acute and chronic hepatitis, cirrhosis and liver with non specific features. Portal tract inflammation and abnormal liver architecture were found in almost all diagnosed cases of Wilson's disease. The most commonly correlated cases (34 cases) included 5 cases of chronic active hepatitis, 4 cases each of Wilson's disease and cirrhosis of liver, 3 cases each of Extra-hepatic portal vein obstruction, acute hepatitis and massive liver cell necrosis.

Conclusion: Liver biopsy can help in giving a definitive diagnosis in most of the cases and can be valuable in cases with clinical overlap.

KEYWORDS : Liver biopsy, Wilson's disease, histopathology, clinico-pathologic correlation

INTRODUCTION

Hepatic and hepatobiliary diseases are a common cause of morbidity and mortality in children. Certain hepatobiliary diseases are confined to the pediatric age group. On one end of the spectrum are viral infections which may be acute and self limiting or may lead to chronic and life-long sequelae while on the other end are metabolic disorders involving the liver like Gaucher's disease, mucopolysaccharidoses, gangliosidosis and galactosemia requiring specific investigations for diagnosis.[1,2]

The age groups that the liver diseases involve in children is also expansive with neonates affected by sepsis related cholestasis, biliary atresia, metabolic disorders while diseases like Wilson's disease present in older age group of children.[3]

Liver biopsy combined with clinical data can suggest a cause in most cases. Specimen of liver tissue can be used to provide precise histological diagnosis in patients with neonatal cholestasis, chronic active hepatitis, metabolic liver disease, intrahepatic cholestasis (paucity of bile ducts), congenital hepatic fibrosis, for enzyme analysis, for analysis of stored material such as iron, copper or specific metabolites, to monitor responses to therapy, to detect complications of treatment with potentially hepatotoxic drugs including aspirin, antimetabolites, antineoplastic or anticonvulsant agents.

Thus, liver biopsy is the choice of investigation in case of uncertainty about the diagnosis and etiology of chronic liver disease.

With this in mind, the aim of our study was to study the clinical profile, evaluate the findings in various liver and hepatobiliary diseases on liver biopsy in pediatric patients (age group 4-15years). Also, to correlate the histopathological findings of liver biopsy with the possible clinical presentation in chronic liver disease, to assess the prognosis, outcome and effects of treatment of chronic liver disease proven by biopsy.

MATERIALS AND METHODS

After obtaining institutional ethics committee approval, this study was conducted over a period of 5 years where all liver biopsies and selective autopsy cases of children between 4-15 years of age were included. The autopsy cases selected were those where the child had complaints related to liver disease, showed signs suggestive of underlying liver disease and investigations revealed altered liver function eg. raised serum enzymes, altered PT/PI etc.

Wilson's work up included tests like S.ceruloplasmin, 24 hour urinary copper, post penicillamine 24 hour urinary copper, Kayser Fleischer ring, dry weight of copper, MRI Brain and family screening. The liver biopsies were stained with hematoxylin and eosin (H &E) stain. Also reticulin stain was done in all cases.

On liver biopsy, the following histopathological features were evaluated:

1. No.of portal tracts (for adequacy)

2. Hepatocytic degeneration

a. Fatty Change (large cytoplasmic droplets of fat causing displacement of cell nuclei).

b. Hydropic degeneration (single or groups of hepatocytes with granular but pale staining cytoplasm compared with normal).

c. Feathery degeneration (cells having faintly staining cytoplasm which has a reticulated appearance and is usually bile stained occurring in groups near portal tracts).

3. Hyaline degeneration [cytoplasm of affected hepatocytes containing perinuclear hyaline bodies (Mallory bodies) of rounded or irregular shape].

4. Hepatic necrosis

a. Zonal necrosis (same zone of each liver lobule is affected).

b. Bridging necrosis (bands of necrotic tissue passing between adjacent portal tracts or centrilobular zones or traversing from individual lobules from center to periphery).

c. Massive necrosis or sub-massive necrosis (necrosis of large area of liver or at least several adjacent lobules in their entirety).

d. Focal necrosis (single necrotic hepatocytes/pin point necrosis)
e. Piecemeal necrosis (loss of cells in limiting plate and adjacent parenchyma in the periportal zones).

5. Hepatocyte inclusion bodies [These may be aggregates of virus (nuclear/cytoplasmic) or stored products of an inherited metabolic disorder].

6. Giant cell transformation (large multinucleated liver cells).

7. Hepatic pigmentation (Bile pigment, lipid pigment, hemosiderin pigment, copper pigment) which may require special stains for confirmation.

8. Liver parenchymal regeneration (plates of hepatocytes more than one cell thick).

9. Hepatitis and cholangitis (Infiltration of liver with acute or chronic inflammatory cells).

10. Hepatic fibrosis (distinguished from true cirrhosis by persistence of a recognizably normal lobular pattern).

11. Cirrhosis of liver (loss of normal liver architecture and parenchyma consisting of hyperplastic nodules, separated by fibrous septa).

RESULTS

57 cases (45 biopsy samples and 12 autopsy cases) between the age group of 4-15 years were studied. The majority of the cases were in the 4-7 year age group which constituted 49.1% of the cases. There was no significant sex predominance with male:female ratio being almost 1:1 **[Table 1]**. The most common presenting feature was jaundice seen in 64.9% of cases followed by abdominal distension (42.1%), fever (36.8%) and hematemesis (12.2%). Anemia was present in 28.8% cases.

There were 11 children (19.3%) who had a past history of liver disease in the form of jaundice, extra-hepatic portal vein obstruction (EHPVO), hepatic encephalopathy or known case of chronic liver disease. The most common abnormal biochemical result was an increase in the level of bilirubin with increased total bilirubin and direct bilirubin seen in 81.5% and 81.08% of cases in which it was performed. This was followed by increased levels of aspartate and alanine transaminase (SGOT/SGPT) seen in 77.2% of cases.

In 4 cases in which hepatic doppler was performed, useful information was obtained in 3 cases, one each of Budd-Chiari syndrome, cirrhosis of liver and non- cirrhotic portal fibrosis (NCPF). Auto-immune markers were carried out in 5 patients which included tests like ANA, ASMA and Anti-LKM-1 antibody. Of these 1 patient showed positive antibody markers. Viral markers were positive in 7 patients (3-Hepatitis A, 2-Hepatitis-B and 2-Hepatitis C). Of the 3 cases with positive hepatitis A markers, 1 had histopathological diagnosis of acute hepatitis. 2 cases were histopathologically diagnosed as cirrhosis of liver with possibility of extra hepatic biliary obstruction (EHBO) and autoimmune hepatitis respectively being the cause for cirrhosis.

There were 7 cases diagnosed as metabolic liver disease on histopathology. Diagnosis of metabolic disorder was given in 5 cases in the 4-7 year age group. This included 3 cases of Wilson's disease and one each of alpha-1 anti-trypsin deficiency and metabolic disorder (not further classifiable). Other 2 cases were seen in the 8-11 year age group which included 2 Wilson's disease cases. Jaundice was the most common clinical feature in patients with Wilson's disease closely followed by splenomegaly and fever

S.ceruloplasmin was positive in 6 out of 15 patients tested (40%), 24 hour urinary copper was positive in 8 out of 11 patients tested(72.7%), post-penicillamine 24 hour urinary copper was positive in 1 out of 3 patients tested(33.3%), dry weight of copper was positive in 2 out of 3 patients tested(66.6%), K-F ring was seen in 2 out of 12 patients (16.6%) **[Table 2]** Out of 6 patients with low serum ceruloplasmin levels, 2 patients were diagnosed finally as Wilson's disease. The other 4 patients with low serum ceruloplasmin levels had non- specific features on microscopy.

The most common histopathological diagnosis seen in our study included acute and chronic hepatitis, cirrhosis and liver with non specific features. Portal tract inflammation and abnormal liver architecture were found in almost all diagnosed cases of Wilson's disease on microscopy **[Table 3]**. Other histopathological findings in the diagnosed cases of Wilson's disease included ballooning degeneration, cholestasis, bridging fibrosis and cirrhosis **(Fig.1a).**

4 cases which were clinically diagnosed as Wilson's disease turned out to be Wilson's disease on histopathology. The other case which presented with frank hepatic encephalopathy turned out to be Wilson's disease on microscopy. Orcein positivity for copper associated protein was seen in 3 out of 5 histopathologically diagnosed Wilson's disease cases.

Out of the 57 cases, 34 cases (59.6%) correlated clinically with the final histopathological diagnosis **[Table 4].** The most commonly correlated cases included 5 cases of chronic active hepatitis, 4 cases each of Wilson's disease and cirrhosis of liver, 3 cases each of Extra-hepatic portal vein obstruction **(Fig.1b)**, acute hepatitis and massive liver cell necrosis **(Fig.1c)**. The other cases included cases of Alagilles's syndrome, autoimmune hepatitis, sclerosing cholangitis etc. In the patient with Alagille's syndrome, liver biopsy showed intrahepatic and canalicular cholestasis with patchy pseudoxanthomatous transformation of hepatocytes and paucity of intrahepatic bile ducts **(Fig.1d)**. We had one patient of Budd-Chiari syndrome. 3 cases showed massive liver cell necrosis on microscopy out of which 2 cases were HBsAg positive and one case was HCV positive.

DISCUSSION

Liver diseases in children comprise a unique and complex group of disorders. With increasing age, the pattern begins to resemble that of adult liver disease, though some differences are observed. Thus, though viral hepatitis in adults and children has similar clinical and pathological manifestations, the risk of developing fulminant hepatitis and chronicity is less in children.[4] Inborn errors of metabolism, toxins, atresias and various heredofamilial disorders account for many causes of childhood cirrhosis.

The mean age of presentation of liver disease in children is almost the same i.e. 4-7 years in literature because metabolic liver diseases manifest earlier, infective diseases in the school going child and Wilson's disease at a later age.[5]

Dhole et al[6] in their study on chronic liver diseases in children found jaundice (73%) and abdominal distension (51%) to be the most common clinical presentation which is comparable to the clinical presentation in our study. Other studies done by Mehnaz et al[7] and Qureshi et al[8] showed anemia to be the most common presenting symptom seen in 75% to 95% of the cases respectively as compared to only 28.8% of cases presenting with anemia in our study.

Jaundice when present with abdominal distension indicates an advancement in liver disease and is generally known to be associated with a poor prognosis. The co-existence of both these symptoms denotes hepatic decompensation as indicated by Grade C of Child's grading of cirrhosis. Even if these patients are subjected to liver transplant, there is a high mortality associated.[9] Anemia is a significant disturbance along with chronic liver disease, the cause being bleeding tendency, poor nutrition and chronic blood loss because of portal hypertension. Anemia can also be immunologically mediated due to antibodies causing injury to RBC's by toxic material like excessive copper and nutritional

deficiency of iron, vitamin B12 and folic acid deficiency.[7,8,10]

High levels of bilirubin was the most common abnormal biochemical result in our study. This is in contrast to studies done by Qureshi et al[8] and Malik et al[10] which showed hypoalbuminemia to be the most common abnormal biochemical parameter seen in 90% and 83% of cases respectively which was seen in only 66.6% of our cases. Their studies also showed abnormal prothrombin time levels in 66 to 90% of cases which corresponds to findings in our study.

Hepatic Doppler was also useful in our study. Findings of a Doppler study become a very useful adjunct at the time of histopathological diagnosis especially in conditions like Budd-Chiari syndrom[11] and non- cirrhotic portal fibrosis[12].

Diversity in the presentation of clinical and serum biochemical features of autoimmune hepatitis in children can lead to a variety of differential considerations. In the case of an acute hepatitis syndrome, acute viral hepatitis and Wilson's disease should be excluded before starting immunosuppressive treatment. Other differentials to be considered include chronic viral hepatitis especially hepatitis B and C, sclerosing cholangitis and toxic or drug induced hepatitis.[13]

Histopathological examination is the gold standard in chronic hepatitis C for assessing changes after anti-viral therapy and is considered mandatory for grading (necroinflammatory activity) and staging (fibrosis) in most patients, including patients with persistently normal aminotransferase values and also for evaluating steatosis, all histopathological features that affect the natural history and therapeutic outcome. In chronic hepatitis B, the same applies.[14]

Though Wilson's disease is usually diagnosed in the 2nd decade of life, the earlier presentation in our study could possibly be because of an insult due to viral hepatitis although WD has been reported even unde 5 years of age with atypical manifestations.[15,16] The ceruloplasmin level is low in patients with Wilson's disease and in heterozygous patients with aceruloplasminemia. In this latter category, there is no associated abnormal copper-related liver pathology. However, the hepatocytes have a marked increase in iron content. The ceruloplasmin level is also low in newborns, infants during the first 6 months of life, patients with protein losing enteropathy, and patients with liver cirrhosis of other causes.[17] Also literature mentions that normal levels of ceruloplasmin or a borderline low ceruloplasmin does not rule out Wilsons disease.[18]

The presence of Kayser-Fleischer (K-F) ring was looked for in 12 patients including 5 patients finally diagnosed as Wilson's disease. Only 2 patients had presence of K-F ring on slit lamp examination. However, none of the patients with Wilson's disease had K-F ring. This could be due to the fact that K-F rings are reported to be usually absent I children presenting with liver disease.[19] Also, K-F like ring has been reported in many other conditions like chronic active hepatitis, neonatal hepatitis, cryptogenic cirrhosis, primary biliary cirrhosis, cholestatic disorders, hepatocellular disorders with bilirubin > 20mg/dl and intra-ocular foreign body containing copper.[20] Kayser-Fleischer ring is present in 98% of patients with neurological manifestations of Wilson's disease but is present in only 44-62% of patients with liver disease manifestations.[17,19]

Orcein is an excellent stain to detect the presence of copper in all cases of Wilson's disease except in the early stages. Timm's silver stain[21] has been seen to be the most sensitive method for the demonstration of copper in all stages of Wilson's disease in a study but was not performed due to non-availability of the stain.

Thus, a constellation of tests along with evaluation of clinical parameters only can help in reaching the appropriate diagnosis of WD since liver morphology is non-specific and copper histochemistry may lead to both false positive and false negative results, the pathologist usually only suspects the disease or assists in its confirmation.[22]

One of the objectives of our study also included the correlation of the histopathological diagnosis with the clinical presentation. Out of the 57 cases, 34 cases (59.6%) correlated with the final histopathological diagnosis. The most commonly correlated ones included cases of chronic active hepatitis, WD and cirrhosis of liver, extra-hepatic portal vein obstruction, acute hepatitis and massive liver cell necrosis. 4 cases clinically diagnosed as Wilson's disease turned out to be Wilson's disease on microscopy as mentioned earlier. In the patient with Alagilles syndrome, liver biopsy showed intra-hepatocytic and canalicular cholestasis with patchy pseudoxanthomatous transformation of hepatocytes and paucity of bile ducts (Fig.2). Here the bile acid levels decreased dramatically after treatment with cholestyramine and phenoparbitone confirming the diagnosis of Alagilles syndrome.

We had 3 clinically suspected cases of extrahepatic portal vein obstruction diagnosed on microscopy. The patients came with complaints of abdominal pain, vomiting and hematemesis. Microscopy showed mild increase in connective tissue mainly around the portal vein radicals and multiple channels in the portal areas with spaces resembling portal veins directly abutting the hepatic parenchyma confirming the diagnosis of extrahepatic portal vein obstruction. It should be noted here that it is best to know the clinical features of the case before rendering a diagnosis of extrahepatic portal vein obstruction because the subtlety of the findings may lead one to report the biopsy as normal even in the face of known portal hypertension.

The bad prognostic features in liver disease include massive liver cell necrosis, cirrhosis and chronic active hepatitis. 15 of our patients had the following features on microscopy, thus having a bad prognosis.

As ours is a tertiary care general hospital, several of our patients were belonging to outside city limits and even across states. It was thus a difficult task to maintain contact with the families of these children with liver disease except in some cases. However, follow up would have idealized the study more. All the 5 cases of Wilson's disease were treated and showed positive response to D-penicillamine.

CONCLUSION

A spectrum of liver diseases can affect the school going child. Liver biopsy can help in giving a definitive diagnosis in most of the cases and can be valuable in cases with clinical overlap. A high index of suspicion along with a clinico-radio-pathological approach can help in diagnosis and treatment of these children.

TABLES:

TABLE 1: DEMOGRAPHIC STATISTICS

Sex / Age	Male	Female	Total
4 - 7 years	13	15	28
8 - 11 Years	10	3	13
12 - 15 Years	6	10	16
Total	**29**	**28**	**57**

Table 1: There appears to be a clustering of cases in the 4-7 year age group. However there is no significant sex predominance.

TABLE 2: Wilson's work up

Investigation	Performed in patients	Abnormal results obtained in
S. Ceruloplasmin	15	6 (40 %)
24 hour urinary Cu	11	8 (72.7 %)
Post Penicillamine 24 hour urinary Cu	3	1(33.3 %)
KF Ring*	12	2 (16.6 %)

Dry weight of Cu	3	2 (66.6 %)
MRI Brain	1	1 (100 %)
Family Screening	12	4 (33.3 %)

**Kayser Fleischer ring*

Table 2: Abnormal results were obtained in only few diagnosed patients of Wilson's disease. S.ceruloplasmin was low in 6 patients of which only 2 patients were diagnosed as Wilson's disease.

Table 3: Histopathological findings in Wilson's disease

Histopathological findings	Positive	Negative
Portal tract inflammation	5	0
Abnormal Liver Architecture	4	1
Ballooning	3	2
Cholestasis	3	2
Macrovesicular Steatosis	1	4
Bridging fibrosis	3	2
Glycogenated nuclei	1	4
Cirrhosis	3	2

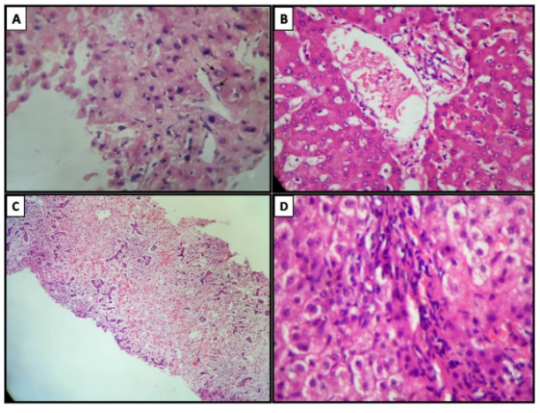
Table 3: There was a very wide spectrum of features seen in Wilson's disease with portal tract inflammation being the commonest.

Table 4: Distribution of Clinical and Histopathological correlated cases

Histopathological diagnosis	Frequency	Percentage
Chronic Active hepatitis	5	14.7
Wilson's Disease	4	11.8
Cirrhosis of liver	4	11.8
EHPVO	3	8.9
Acute Hepatitis	3	8.9
Massive Liver Cell Necrosis	3	8.9
Focal Necrosis	1	2.9
NCPF	2	5.9
Alagille's Syndrome	1	2.9
Liver Necrosis in Typhoid	1	2.9
Non Specific Features	2	5.9
Sclerosing Cholangitis	1	2.9
Autoimmune Hepatitis	1	2.9
Liver Abscess	1	2.9
Metabolic Disorder	1	2.9
Budd-Chiari Syndrome	1	2.9
Total	34	100.0

Table 4: The most common correlated cases included those of Wilson's disease, chronic active hepatitis, cirrhosis and acute hepatitis.

FIGURE 1:



LEGENDS FOR FIGURES:

Fig.1: A. Wilson's disease showing classical features of intrahepatocytic cholestasis, macrovesicular steatosis and glycogenated nuclei (marked with arrows). (H&E,400X)

B. Dilatation of the portal vein in Extra-hepatic portal vein obstruction (EHPVO). (H&E,400X).

C. Massive liver cell necrosis. (H&E, 40X).

D. Alagille's syndrome: liver biopsy showing intrahepatocytic and canalicular cholestasis with patchy pseudoxanthomatous transformation of hepatocytes and paucity of intrahepatic bile ducts. (H&E,400X).

References:

1. Cochran JB, Losek JD. Acute liver failure in children. Pediatr Emerg Care. 2007; 23(2): 129-35.
2. Tahir A, Malik FR, Ahmad I, Akhtar P. Aetiological factors of chronic liver disease in children. J Ayub Med Coll Abbottabad.2011;23(2):12-14.
3. Burki MK, Orakzai SA. The prevalence and pattern of liver disease in infants and children in Hazara division. J Ayub Med Coll Abbotabbad.2001;13(1):26-8.
4. Kelly D. Viral hepatitis B and C in children. J R Soc.Med.2006;99(7):353-7.
5. D'Agata ID, Balisteri WF. Evaluation of liver disease in the pediatric patient. Pediatr Rev.1999;20(11):376-90.
6. Dhole SD, Kher AS, Ghildiyal RG, Tambse MP. Chronic liver diseases in children: Clinical profile and histology. J Clin Diagn res.2015;9(7): SC04-7.
7. Mehnaz A. Chronic liver disease in children: an overview. Med Spect 1999;20:7-11.
8. Hanif M, Raza J, Qureshi H, Issani Z. Etiology of chronic liver disease in children. J Pak Med Assoc.2004;54(3):119-22.
9. Kumar and Clark: Child's grading of cirrhosis. Liver, Biliary tract and pancreatic diseases. Clinical Med, 5th Ed 2002, Saunders, Ch.7:365-66.
10. Malik M, Hussain W, Aslam M, Maqbool S. Clinical spectrum of chonic liver disease in Pakistani hospitalized children. Pak Ped J.2000;24:9-12.
11. Chaubal N, Dighe M, Hanchate V, Thakkar H, Deshmukh H, Rathod K. Sonography in Budd-Chiari syndrome. J Ultrasound Med.2006;25:373-9.
12. Sinha R. Grayscale and pulsed Doppler characteristics of non-cirrhotic portal fibrosis: a preliminary report. Clin Radiol.1999;54(3):156-9.
13. Nanda Kerkar, Cara L.Mack. Hepatitis and immune disorders: Autoimmune hepatitis. In: Frederick J.Suchy, Ronald J.Sokol, William Ballisteri, editors. Liver disease in children.4th edition. Cambridge University Press;2014.p.311-21.
14. Cholongitas E, Senzolo M, Standish R, Marelli L, Quaglia A, Patch D et al. A systematic review of the quality of liver biopsy specimens. Am J Clin Pathol.2006;125(5):710-21.
15. Beyersdorff A, Findeisen A. Morbus Wilson: Case report of a two-year old child as first manifestation. Scand J Gastroeneterol.2006;41(4):496-7.
16. Kalach N, Seidman EG, Morin C, Rasquin-Weber A, O'Regan S, Laberge JM et al. Acute liver failure from Wilson's disease in a five year-old child. Can J Gastroenterol.1993;7:610-2.
17. Mounif EL, Youssef MD. Wilson's disease Review. Mayo Clin Proc.2003;78:1126-36.
18. Weiss KH. Wilson disease.1999 (updated 2016). In: Pagon RA, Adam MP, Ardinger HH et al., editors. Gene Reviews (Internet). Seattle (WA): University of Washington, Seattle;1993-2016.
19. Roberts EA, Schilsky ML. Diagnosis and treatment of Wilson's disease: an update. Hepatology 2008;47(6):2089-2111.
20. Suvarna JC. Kayser Fleischer ring. J Postgrad Med.2008;54(3):238-40.
21. Langner C, Denk H. Wilson's disease review article. Virchow's Arch.2004;445:111-8.
22. Pilloni L, Lecca S, Van Eyken P, Flore C, Demelia L, Pilleri G et al. Value of histochemical stains for copper in the diagnosis of Wilson's disease. Histopathology.1998;33(1):28-33.