Research Article

The clinicopathological correlation in suspected cases of chronic liver disease with the aid of liver biopsy – a study in tertiary health centre

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Abstract

Background: Hepatic disorders are prevalent in all ages. They are challenging to both pathologist as well as clinician in view of the ill-understood etiopathogenesis. Histological assessment of the liver should be performed and thus, liver biopsy is a cornerstone in the evaluation and management of patient with chronic liver disease.

Objective: To study histological spectrum in patient of chronic liver diseases, correlate clinical and biochemical features with morphology on liver biopsy, and to evaluate the role of liver biopsy in the diagnosis and management of chronic liver disease.

Materials and Methods: This study is based on liver biopsies obtained from 62 patients with suspicion of chronic liver disease based on clinical features and findings on other investigations. Relevant biochemical and other investigations were done before liver biopsy was resorted. The size of the intact liver biopsy obtained are ranged from 1.5 to 2.5 cm, though fragmented biopsy was also obtained in certain cases and was subjected to routine H and E staining and special stains in some cases were performed.

Result: Out of 62 biopsies performed, fatty liver due to non- alcoholic steatohepatitis (NASH) was seen in 27% of cases followed by alcoholic cirrhosis in 19% of cases while least case was found due to hepatitis C in only 03% cases. Males were affected more.

Conclusion: Liver biopsy study is the gold standard for diagnosis of chronic liver diseases.

KEYWORDS: Liver biopsy, chronic liver diseases, clinicopathological correlation

Introduction

The clinical entity of chronic liver disease is difficult to define. Generally, vague discomfort in abdomen associated with indigestion, flatulence and pain or heaviness in the right hypochondrium are the symptoms that may have some relation to liver disease. To clinician, hepatomegaly or symptoms referable to the liver with or without jaundice and biochemical abnormalities for six months or above suggests a probable chronic liver disease. According to Sherlock[1], any liver disease which is existing for more than 6 months is taken as chronic liver disease.

A large number of etiological factors have been postulated in the causation of chronic liver diseases. These in order of frequency are: chronic hepatitis C, alcoholic liver diseases, non-alcoholic steatohepatitis, chronic hepatitis B, autoimmune hepatitis, drug induced hepatitis, primary biliary cirrhosis, haemchromatosis, and Wilsons disease.[2–6]

Historically, liver biopsy was used almost exclusively as a diagnostic tool. The three major roles of liver biopsy are: (a) For diagnosis. (b) To assess the prognosis (disease staging) and (c) To assist in making therapeutic management decisions.[7–13]

This study aims at exploring the role of histopathological studies of biopsied liver tissue in the management of patients

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suffering from chronic liver disease suspected on the basis of clinical, biochemical, and serological parameters. The histomorphological study of liver biopsy probably is and may remain the best means to ascertain the nature and significance of pathological processes occurring in the liver.

Materials and Methods

This study is based on liver biopsies obtained from 62 patients with suspicion of chronic liver disease based on clinical features and findings on other investigations. The study period was 18 months and was carried at Dr. D Y Patil Medical College, Pune. Relevant biochemical and other investigations were done before liver biopsy was resorted to. History suggestive of liver disease like jaundice with duration and recurrence were asked for. History of injections, infusions, and use of hepatotoxic drugs were also sought. Physical examination included assessing obesity (body mass index), pallor, edema or ascitis, hepatomegaly, spenomegaly, and others. Investigations included blood parameters, liver function tests including enzyme estimations, blood glucose levels. Bleeding time, clotting time, platelet counts, and prothrombin time were estimated before liver biopsy was attempted.

Liver biopsy was done after explaining the procedure, its benefits, limitations, and drawbacks to the patient to obtain informed consent. Intercostal approach was used with appropriate local anaesthesia. VimSilvermann needle was used in most cases while in others liver gun was used. The size of the intact liver biopsy obtained was ranged from 1.5 to 2.5 cm though fragmented biopsy was also obtained in certain cases.

As soon as the needle biopsy was obtained from the patients, it was expelled gently onto a piece of filter paper. This was done to prevent distortion of fragmentation of the tissue. Biopsied tissue was fixed in 10% formal saline overnight and processed by the following schedule which took 15 hours.

Thereafter, sections of average 5 μm thicknesses were made. In all cases the sections were stained by the following methods: haematoxylin and eosin, silver impregnation for reticulin fibres, Masson's trichrome stain, and Pearls stain.

Result

Liver biopsy showed fatty liver due to non-alcoholic steatohepatitis (NASH), and was found in maximum number of cases that is 27%. Chronic liver disease due to alcoholic cirrhosis was found in about 19% of cases accounting for second most cause of chronic liver disease. Fatty liver due to alcohol, alcoholic hepatitis and cirrhosis accounted for third most common cause of chronic liver disease in the present study found on liver biopsy. While rest of the causes for chronic liver disease are depicted in Table 1.

Most patients belonged to the age groups of 40–50 years and above 60 years constituting 26 (42%) in each group. Patients in the age group of 20–39 years were 8 (13%), while 2 (3%) were between 0 and 19 years of age. Male female ratio was 2.4:1. Males were more than females in this study.

Table 1: Histopathological diagnosis

Histopathology diagnosis	Number of cases	%
Fatty Liver		
Alcoholic	06	10
NASH	16	27
Hepatitis		
Alcoholic	06	10
Hepatitis B	04	06
Hepatitis C	02	03
Cirrhosis		
Alcoholic	12	19
Posthepatic	02	03
Unknown cause	06	10
Neoplastic		
Primary (HCC)	04	06
Non-specific changes	04	06

Dull aching abdomen pain was present in upper right hypochondrium in 74% of patients with chronic liver disease accompanied by tenderness over liver area. Nearly half of the patient (45%) complained of nausea which was provoked by odour of food or by intake of fatty food.

Jaundice was the most reliable marker of severity of the disease which was best demonstrated by scleral icterus. Mild to moderate jaundice ranging from 2 to 12 mg/dl bilirubin was observed in 32% of cases. One had severe jaundice where bilirubin was more than 20 mg/dl. Hepatomegaly and ascitis were detected by clinical and ultrasound examination.

Increased bilirubin levels ranging from 2 to 25 mg/dl were present in 32% of cases. Alanine aminotransferase (ALT) levels were elevated in 68% patients. Aspartate aminotransferase (AST) levels were elevated in 74% cases. Gamma globulin levels are increased in chronic liver diseases. Increased levels were found in 13% of our cases. Raised alkaline phosphatase was present in only one (03%) case. HBsAg positive individuals were 16% while, anti-HCV antibody was detected in 2 (03%) patient reflecting the phase of the disease.

As NASH was found in maximum number of cases staging and grading of NASH (criteria of Brunt) was done given in Table 2. The patients having NASH had increased BMI>28, were diabetic and had increased or borderline cholesterol levels. The histopathological diagnosis of NASH included steatosis with ballooning degenerative was seen from zone 1 to zone 3 with lobular inflammation comprising of predominantly neutrophils or lymphocytes in different patients. While, 1 case had lipogranulomas with inflammatory cell comprising of eosinophils, lymphocytes, and gaint cells. Perisinusoidal fibrosis was noted.

In alcoholic liver disease AST > ALT was found in all cases. The histopathological results found on liver biopsy showed macrovesicular to microvescicular steatosis seen distinctly

Table 2: Staging and grading of NASH (criteria of Brunt)

Grading	No. of patients	%
Mild (Grade 1)	8	50
Moderate (Grade 2)	6	38
Severe (Grade 3)	-	-
Staging	**No. of paients**	**%**
Stage 1	2	12
Stage 2	6	38
Stage 3	-	-
Stage 4	-	-

from zone 1 to zone 3 in different cases. While in only one case, megamitochondria was found. Distorted architecture, with well formed nodules surrounded by vascular septae was also seen in few cases.

In hepatitis B (HB), histopathology (with Ishak grading and staging) enlarged the hepatocytes with cytoplasmic finely granularity, pale eosinophilic material filling cytoplasm with spotty lymphocyte infiltrate around portal area with destruction of limiting plate. Interface hepatitis involves most of the portal area with ballooned hepatocyte and inflammatory cell aggregate.

In hepatitis C (HC), traids of lymphoid aggregate in portal traid, steatosis with bile duct injury were found. Mild interface hepatitis was noted with evidence of cholestasis. No fibrosis was seen. While in hepatocellular carcinoma trabecular pattern of growth with exaggerated liver plates 15–20 cells thick were separated by sinusoids. Bile stasis was also noted.

Discussion

Chronic liver disease is an important cause of silent morbidity and mortality worldwide. The correct and early diagnosis of the condition can ensure better prognosis. The common presentation of chronic liver disease in this study was abdominal pain with hepatomegaly occurring in 40–60years patients with male preponderance. Derangement of one or more of the LFT was observed in all the cases. Out of 62 samples biopsied, the exact diagnosis was offered in 58 cases (94%). NASH constituted the maximum number of cases amongst the biopsies performed (27%). Histologically, lobular inflammatory cells comprised of neutrophils, lymphocytes, eosinophils, and giant cells along with lipogranuloma formation. In alcoholics, AST:ALT ratio was more than 2:1 in most of the cases. End stage alcoholic cirrhosis was seen in 19% of the cases. Histologically, alcoholic steatohepatitis was dominated by polymorphonuclear infiltrate. Megamitochondria were also observed. Chronic viral hepatitis was seen in 19% of cases. HB was 83% of the cases and HC 16%. Ishak grading and staging was used for chronic hepatitis. This helped to assess the fibrosis progressing to cirrhosis and had significant prognostic implications.

According to Gao et al.[14], world health statistics 2007–2009 indicate that chronic liver disease occurs in age group of more than 55 years with steady increase in death rate up to 75+ years. In this study, majority of the individuals suffering from chronic liver disease were in 40–60 years of age with male preponderance. Wang et al.[15] and Gelb et al.[16] also reported male predominance in their study. While in a study by Drebber et al.[17], it was found that females were affected more than males. In this series 74% of cases complained of abdominal pain with discomfort while 42% had hepatomegaly clinically. In another series, 10% patients had abdominal pain while 48% patients had hepatomegaly.[18] Biopsy performed by Laurin[18] found 2–3% cases of NASH while in this study 27% cases had NASH diagnosed on liver biopsy after excluding viral, metabolic, autoimmune, and genetic causes of chronic liver disease. A study by Kanemasa and Sumida[19] showed that 35% cases of NASH had elevated ALT levels while Fracanzani et al.[20] found that increased ALT levels were observed in 74% of their cases. In the present study, 50% patients had elevated ALT levels. In present study it was found that histopathologically steatosis and ballooning were constant findings in all cases. While lobular inflammatory cells comprised mostly of neutrophils and lymphocytes, in one case eosinophils along with gaint cells were also present. Lipogranuloma was seen in this case due to rupture of lipid vacuole. Studies by Ratizu et al.[21] and Kanemasa and Sumida[19] showed presence of polymorphs and lymphocytes only. Fibrosis was well seen on H and E in 2 out of 3 cases, while in one case where the biopsy was fragmented reticulin stain was useful in demonstrating fibrosis. A study done by Raszeja-Wyszomirska[22] showed that cirrhosis development was 20% in NASH patients while in this study no cirrhosis was found in NASH patients. Brunt grading and staging of NASH was used in this study because it defined the grading criteria as mild, moderate, and severe under heading of steatosis, ballooning, portal, and lobular inflammation numerically, while staging which included fibrosis was mentioned separately.[23] This was also used by different authors.[21,24] In a tertiary health centre, most of the alcoholic patients presented in end stage liver disease when their management becomes difficult in primary and secondary health centres. The AST: ALT ratio was more than 2:1 in all biopsied cases of alcoholics. Ratio more than 3:1 is highly suggestive of alcoholic liver diseases as found in other studies.[25,26] PT was raised up to more than 1 s of laboratory control in this study in few patients. In such individuals, parenteral administration of vitamin K was made with normalization of PT levels before biopsy. Male drinking pattern as alcoholics was also observed in other study.[27] Biopsy sample showed fatty liver, alcoholic steatohepatitis in 25% cases each and cirrhosis in 50% of cases in contrast to 33% of fatty liver, 20% alcoholic steatohepatitis and cirrhosis in 25% of the cases in a study by Lelbach.[28] Histopathology of fatty liver showed macrovesicular steatosis in two cases, while mixed steatosis was seen in one case. One case showed foamy degeneration. In steatohepatitis cases polymorphonuclear (PMN) inflammatory infiltrate was present with ballooning

and steatosis. Lelbach[28] also had similar observations in alcoholic hepatitis cases. In some cases of AH megamitochondria were demonstrated. This was not reported by other workers.[29,30] Perivenular fibrosis was seen which again helped to differentiate alcoholic hepatitis from viral hepatitis as generally no perivenular fibrosis is seen in viral hepatitis. Alcoholic cirrhosis was seen in 50% of alcoholics in this study. While Gelb et al.[16] and Menon et al.[25] had 34% and 20% cases of alcoholic cirrhosis, respectively. In contrast to the other two showed active cirrhosis with features of chronic hepatitis, interface hepatitis, and necrosis in the lobules. In a study by Valleneuve et al.,[31] 16 of 31 biopsies performed on HBsAg positive cases, one of the transaminases levels were elevated. In the present study, both of the transaminases levels were raised in HBsAg positive patients. However, Ray et al.[32] found that there was no correlation between HBsAg status and transaminases levels. In one study, it was found that out of 31 HBsAg patients 24 had chronic persistent hepatitis, 2 each had chronic aggressive hepatitis and steatosis while 3 had normal hepatic morphology.[31] In present study, out of 5 HBsAg positive individuals only two had features of chronic aggressive hepatitis. In one Belgian study, about 66% of HB positive patients developed cirrhosis.[33] While in this study only 20% of the HBsAg positive patients had features of cirrhosis. Chronic viral hepatitis was seen in 19% including HB and HC of total biopsied samples with ALT > AST in more than half of the cases. HBsAg was present in all cases of HB. Histopathology showed interface hepatitis and lobular activity in two cases of chronic active hepatitis. Interface hepatitis does not occur uniformly throughout the liver and varies with some portal tracts not showing this change. The suboptimal biopsies may have unchanged architecture or only minimal portal inflammation although the patient may be quite ill with significant increase of transaminase values. Two cases of primary HCC were seen in HBsAg positive individual showing features consistent with malignancy along with bile plugs. No distant metastases were noted. Hepatitis C was observed in one patient of this study histologically showing traid of lymphoid aggregate in portal tract, steatosis, and bile duct injury as was seen by Lefkowitch[34] in his study. Variable macrovesicular steatosis without zonality was observed in contrast to alcoholic steatosis where steatosis is distinctly zonal, initially in zone 3 and then in zone 2. Ishak[34] grading and staging was used for chronic viral hepatitis because it provided detailed information, was excellent for research, grading was fairly reproducible, and confluent necrosis and developing cirrhosis were well delineated. In this study two biopsies with chronic viral hepatitis were graded as 3, showing mild focal interface hepatitis and focal confluent necrosis with lobular activity having ballooned cells with inflammatory cells in one focus or less per 10x objective. In this study, 4 biopsies were reported as showing non-specific changes. One showed sinusoidal dilatation and congestion with few scattered inflammatory cells. The other one as already mentioned earlier, was not the representative tissue of the lesion. Hence, proper selection of the biopsy site, if needed with the use of ultrasound should be made.

Liver biopsy in this study helped us to pinpoint the exact cause of liver dysfunction in most case while due to improper sampling diagnosis was inconclusive.

Conclusion

Chronic liver disease is an important cause of silent morbidity and mortality worldwide. The correct and early diagnosis of the condition can ensure better prognosis. NASH constituted the maximum number of cases (27%) followed by alcoholic liver disease and chronic viral hepatitis (19% each). Liver biopsy study is the gold standard for diagnosis of chronic liver diseases. It is helpful in cases of hepatomegaly with abnormal LFT. It is also useful even in those with normal LFT results. Thus, it forms a major tool for management for such patients.

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