Original Article

Correlation Of Histopathological, Biochemical & Clinical Spectrum Of Chronic Hepatitis B - A New Look In To Old System

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ABSTRACT

Background: Hepatitis B virus infection is a global health problem. Histopathological examination is the mainstay in evaluating the disease progression and designing treatment protocols in these patients. The aim of this study is a correlation of histological features with clinical and biochemical parameters and what new could be proposed for the future by analyzing the limitations of scoring system used.

Methods: We evaluated 195 patients of hepatitis B virus infection over a period of 9 years & analysed their clinical & histological features and correlated them. Histological evaluation was done according to modified Ishak's grading and Scheuer scoring system.

Results: Patients were divided into age groups of < 40 & > 40 years. The majority of the patients were asymptomatic, male sex , younger age & had lower grade and stage. The mean values of total bilirubin and Prothrombin time of patient groups were within the normal ranges. A significant correlation was seen with stage III & IV and presence of HBe antigen & Anti HBe antibody cases [P-value < 0.05].

Conclusions: We concluded that liver biopsy remains the only diagnostic test in evaluating liver pathology in hepatitis B infection. With certain modifications in the current existing scoring systems, histopathology along with clinical & biochemical correlation is essential to plan the proper management of patients.

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Introduction

Hepatitis B virus [HBV] infection is an emerging worldwide health problem among all viral hepatitis. According to *Lavanchy D*[1] there are about 2 billion people who are infected with HBV and 350 million among them are suffering from chronic HBV infection. The culprit of 10th leading cause of death worldwide in 2004 is HBV.[2] But in the present scenario; it is the 3rd most common cause of death.[3] The histopathological changes of hepatitis B virus can reflect the clinical course of the disease.

The salient histopathological features in viral hepatitis B infection are different in various clinical disease scenarios. Few studies noted that seroconversion of HBe Ag to anti-HBe is followed by a significant improvement of disease activity.[4-7] Fong et al[8] documented that persistence of HBsAg, HBeAg and high titre of HBV DNA for more than 6 months implies progression to chronic HBV infection.

The prognosis of chronic HBV infection is determined predominantly by the presence or absence of active viral replication, the degree of histological liver damage by assessing scoring systems (Modified Ishak activity grade & Scheuer system staging) for the degree of liver fibrosis & that of necroinflammatory activity so this retrospective study was planned to see these effects.

Material and Methods

Study Design: There were 195 patients who underwent liver biopsy as per Standard Treatment Protocols following the inclusion & exclusion criteria as described:

Inclusion Criteria: HBs Ag positive for more than 6 months, HBV DNA viral load >2000 copies for HBe Ag negative patients, HBV DNA >20000 copies for HBe Ag Positive patients, No definite evidence of Cirrhosis clinically, Alanine transaminase [ALT]/Aspartate transaminase [AST] Levels >1.5 times of upper limit.

Exclusion Criteria: Co-infection with HCV & HIV infection, significant alcohol intake >40 gm/day, co-existing metabolic diseases like Wilson disease, alpha 1 antitrypsin deficiency, autoimmune hepatitis etc, failure to give consent, coagulopathy with International Normalized Ratio >1.5, Platelet count < 80 x 10^9/l, inadequate liver biopsy.

The study was a hospital based retrospective study & protocol was approved by the institutional ethics committee and written consent for liver biopsy was already obtained from the patients. Detailed Clinical & Biochemical data were analysed. Liver biopsies were graded according to Ishak Modified Histological Activity Index [HAI] grading and staged according to Scheuer staging system. For this, four separate parameters were considered and scores for individual parameters were added to calculate the total score.[9, 10]

Statistical Analysis: All the clinical, biochemical and histological findings were then analysed statistically using Chi-square test, Fisher Exact test, Unpaired t test.

Results

The age varied from seven to seventy years with a maximum number of patients being between 20 - 40 years. Male to female ratio was 5.9:1. The majority of the patients 164 [>80%] were asymptomatic. Others presented with jaundice, pain, discomfort in right hypochondrium and constitutional symptoms like weakness, lethargy, anorexia & easy fatigability. On physical examination, 59 [30.25%] cases had mild hepatomegaly. Some had mild icterus, splenomegaly and ascites. No clinical evidence of portal hypertension seen.

On investigations; 28 patients had anaemia <10 gm/dl. Thrombocytopenia noted in 2 patients. Mild leukocytosis noted in 2 and leucopenia seen in one patient only. Raised serum bilirubin levels in 21 patients with level ranging from 2.6 to 7.3 mg/dl. Patients had AST and ALT levels were more >1.5 times of normal. 110 patients were HBeAg positive while 85 patients were anti-HBe antibody positive.

Histological Findings: All the 195 liver biopsies were graded according to Ishak's Modified grading system & Scheuer Staging system [Figure1, 2].

The first parameter considered was Piecemeal Necrosis. The scores ranged from 0 to 4. A maximum number of patients had 0 score i.e. 115 cases [58.97 %], followed by score 1 in 48 [24.61 %] patients. 16 patients had a score 2 & 10 with 3 while 6 had a score of 4. The second parameter considered was Confluent Necrosis and scores for this ranged from 0 to 6. Maximum cases i.e. 169 [86.66 %] had a score of 0. 8.2 % cases had confluent necrosis of score 1 while the rest few cases had a score of 2 and 3. Score 4-6 was not seen in any of the cases. The next parameter analysed was spotty Necrosis of hepatocytes which were again scored from 0 to 4 depending on the number of these foci seen per 10 x magnification. A maximum number of cases i.e. 110 [56.41 %] had a score of 1. Sixty-five patients had a score of 2; 16 had 3 while 4 with no focus of spotty necrosis. Portal inflammation score ranged from 0 to 4. A maximum number of cases i.e. 101 [51.79%] had a score of 1. Sixty patients had a score of 2, followed by 15 patients of score 3, 9 with a score of 4 and 10 with 0. Scores for individual grade were added and an overall histological activity index was calculated. A maximum number of patients [62%] were with HAI score of 1-3 [minimal activity] followed by

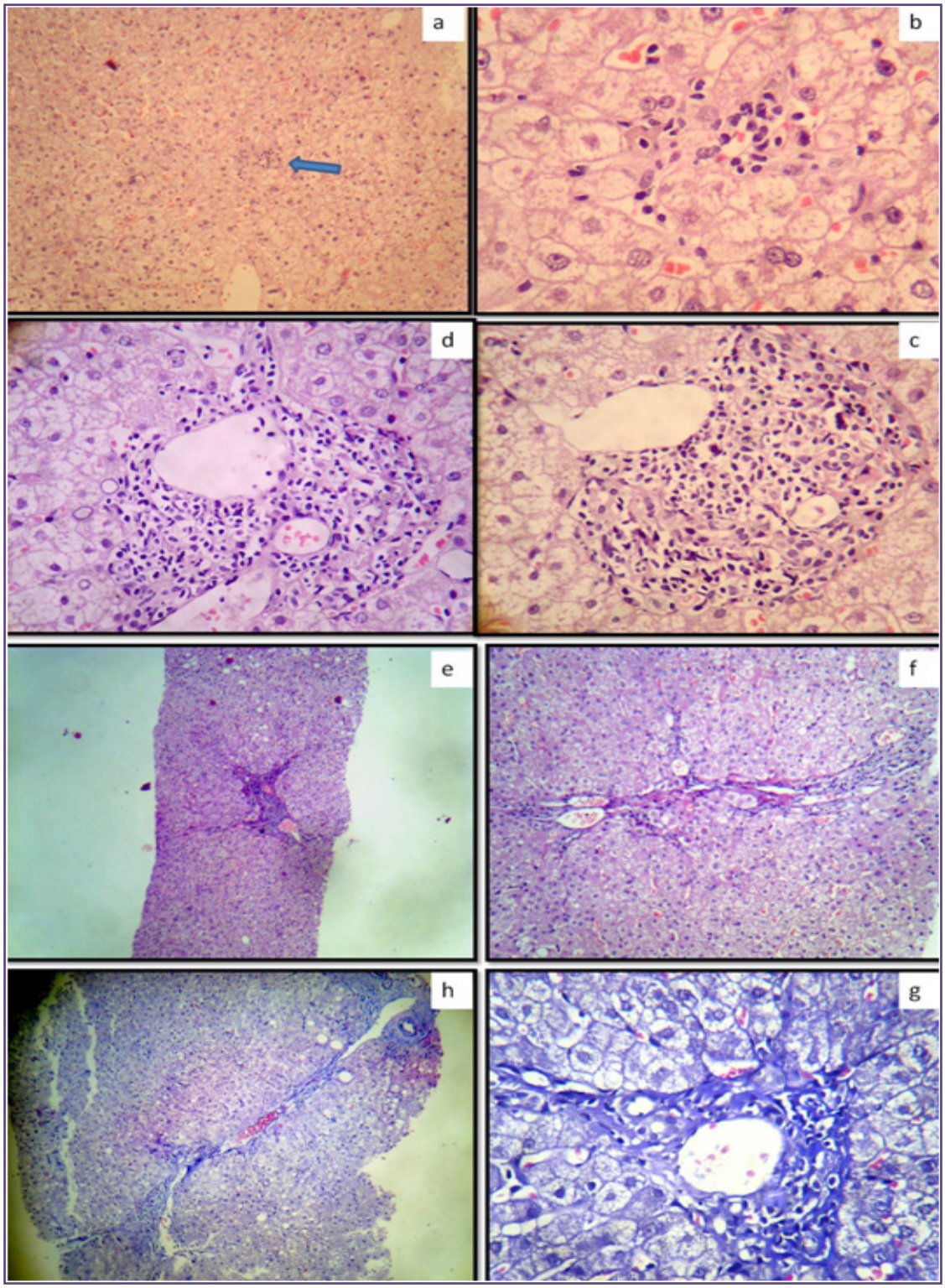

Fig. 1: HAI Score 5, Grade 2, Stage 1, [a & b] A solitary focus of spotty necrosis- arrow [H & E, x 100 & x 400]. [c] Moderate to marked degree of portal inflammation [H & E, x 400]. [d] Portal inflammation with a focus of piecemeal necrosis [H & E, x 400]. HAI Score 3, Grade 1, Stage 2, [e & f] Mild degree of portal inflammation [H & E, x 100 & x 400]. [g & h] Periportal septal fibrosis [Masson Trichrome x 400 & x100].

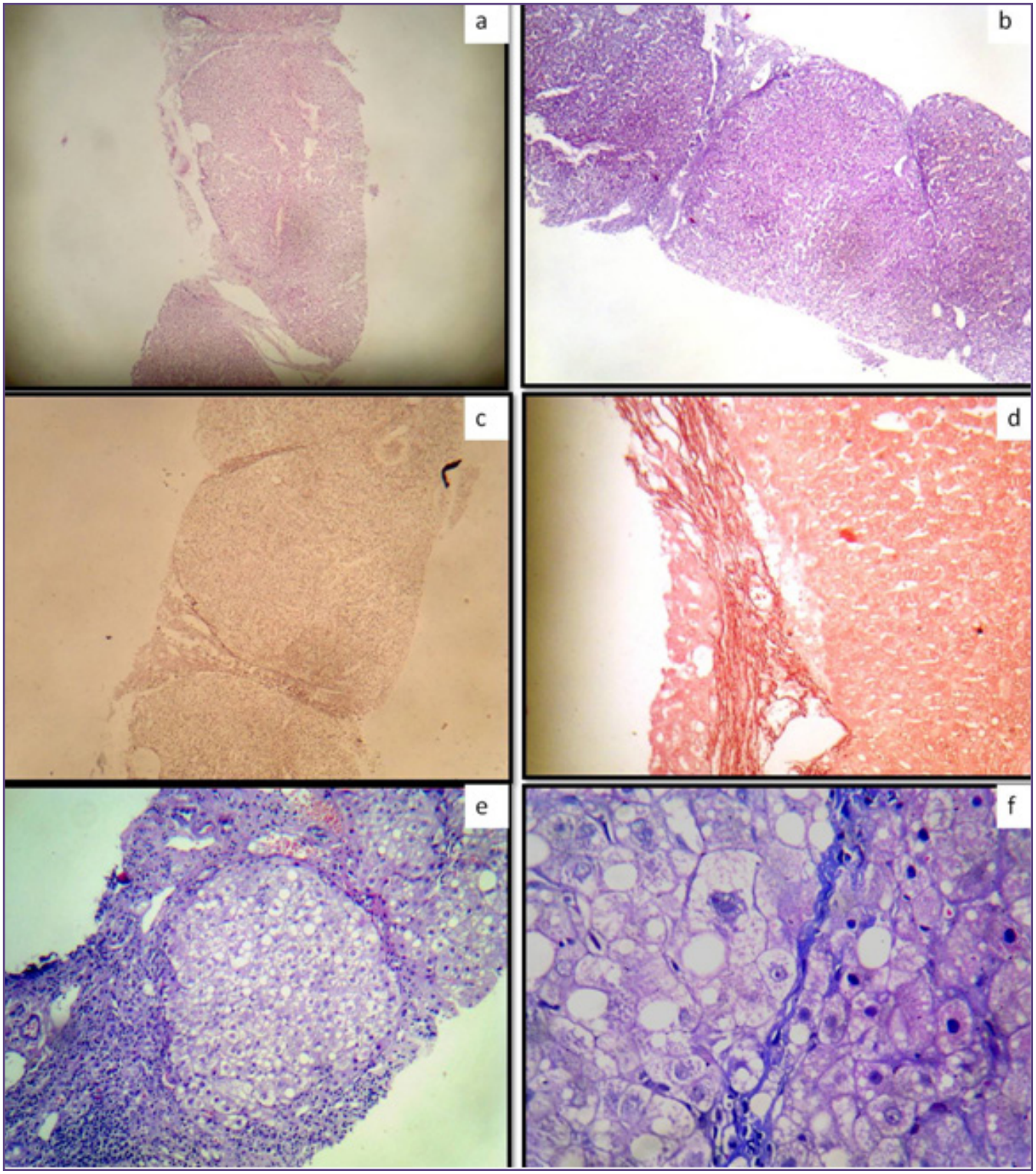

Fig. 2: HAI Score 2, Grade 1, Stage 3, [a & b] Bridging fibrosis look like incomplete nodule formation [H & E, x 100 & Masson Trichrome x100] [c] C shaped fibrosis [Reticulin x 100 [d] Thick fibrous septae indicating old lesion[Orcein x 400]. HAI Score 6, Grade 2, Stage 4, [e] Nodule formation [H & E, x 100].] [f] Collagen bundles [Masson Trichrome x 400].

29.7% with HAI score of 4-8 [mild activity]. HAI score of 9-12 [moderate activity] was seen in 7.2% of cases while least HAI score 13-18 [Severe activity] was observed in only 1% of patients. It means maximum patients were of grade I & II.

Fatty change which was noted in 52.8%, ballooning degeneration in 24.6%, bile ductular proliferation in 2.05% and ground glass appearance of hepatocytes in 17.94% of cases. A maximum number of patients [76 cases] had no increased fibrosis i.e stage 0. Seventy-one case had stage I. Stage II was noted in 25 cases while stage III in 10 patients. 13 cases had Stage IV. HBs Ag was positive in 28 of our cases.

Correlation of Histological Features with Clinical and Biochemical Parameters

Correlation of Grade and Stage with Age [Table 1]: Minimal, mild and severe activity was more common in the age group <40 years and moderate activity in >40 years. Though, statistically, it was not significant. There was no certain trend of age with worsening activity; the observation was not significant on the overall basis. Stage 0, I and II were more common in age group <40 years than in >40 years with a P-value of >0.05 [Not significant]. Advanced fibrosis and cirrhosis were observed more in the age group >40 years than in < 40 years with a P value of >0.25.

Correlation of Grade and stage with Sex & Clinical Features: There were 162 males and 33 females. No significant difference was seen in the distribution of activity and stage with sex [P >0.01].

Clinical features [all together] were correlated with the various grades. It was seen that severity of symptoms did correlate with histological grading & staging [Table 2]. Symptoms could predict the histological outcome. Most of the patients were having a necroinflammatory grade in the form of minimal or mild activity [HAI score 0-8] of which 151 patients with had no symptoms while 17 had jaundice, 9 had constitutional symptoms and 2 had discomfort in right hypochondrium. In moderate to severe activity [HAI score 9-18] category, 13 patients with had no symptoms while 1 each had both jaundice and constitutional symptoms. In stage III & IV of advanced fibrosis and cirrhosis, 14 patients were asymptomatic, 7 had a history of jaundice while 2 had constitutional symptoms. None of the patients included in the study had clinical, radiological & endoscopic evidence of portal hypertension however 23 patients had histopathological features of advanced cirrhosis.

Table 1: Correlation of histological grade and stage with age groups [<40 & >40 years]

Grade / Stages	Group I [< 40 years] [n = 126]	Group II > 40 years [n = 69]	P value / Significance
No activity [Grade 0]	0	0	Chi Square Test P > 0.05 Non-Significant
Minimal activity [Grade I]	82	39	
Mild activity [Grade II]	40	18	
Moderate activity [Grade III]	02	12	
Severe activity [Grade IV]	02	00	
Stage 0,I,II [Mild fibrosis]	116	77	Fisher Exact Test [2 tailed Test] 0.2530
Advanced Fibrosis and cirrhosis [Stage III, IV]	10	12	

Table 2: Correlation of histological grade and stage with presenting symptoms

Grade	With Symptoms [n=31]	No symptoms [n=164]	Statistical Analysis	P value or Significant
No activity	0	0	Chi= 10.55 d.f.=3	Significant [P < 0.05]
Minimal activity	12	109		
Mild activity	14	42		
Moderate activity	3	11		
Severe activity	2	02		
Stage	With Symptoms [n=31]	No symptoms [n=164]		
Stage 0,I,II	22	150	Fisher Exact Test [2 tailed]	P = 0.0035 Highly Significant
Advanced Fibrosis and cirrhosis [Stage III, IV]	9	14		

[d.f. = Degree of freedom]

Correlation of Grade and Stage with AST and ALT Levels: The majority of the patients having minimal activity had these enzyme levels >1.5 to 2 times of normal. However, as there was no certain trend in these enzyme levels and activity, so this observation was not significant on an overall basis. There was also no significant association between the severity of fibrosis and AST & ALT levels [P >0.01].

Correlation of Age, Sex, Symptoms, Grade & Stage with HBe Ag Status: The mean age of HBe Ag-positive patients was 33.56 ± 4 and anti-HBe antibody positive patients 43.3 ± 7 [p-value <0.05]. Males were more in both HBeAg-positive and anti HBe antibody positive chronic hepatitis. The majority of the patients were asymptomatic in HBeAg-positive i.e. 89 patients and 75 of anti HBe positive patients. There was no difference between the two groups with respect to ALT/AST levels. Minimal to mild activity was observed in 104 patients of HBeAg-positive as compared to 75 patients of anti-HBe positive chronic hepatitis. Moderate to severe activity was more common in anti-HBe positive patients i.e. 10 patients as compared to 6 patients with HBe Ag positive patients. Advanced fibrosis and cirrhosis were observed in 18 patients of HBeAg-positive chronic hepatitis as compared to 5 patients of anti-HBe positive chronic hepatitis which were statistically significant [Table 3]. Few of the parameters were not considered in any of the existing scoring systems [Figure 3].

Discussion

No certain trend was found between the severity of activity and age group. However, cirrhosis was commoner in age group >40 years. Similar, observations were also recorded by DiMarco et al[11] & Fattovich et al[12] who in their study encountered age group of 9 years to 60 years with male preponderance. Campbell et al[13] stated that old age is an independent risk factor for progression to cirrhosis. Brunelto et al[14] also emphasized that old age is a well-established predictor for cirrhosis development. Ott JJ et al[15] stated that the pattern of age-specific HBs Ag prevalence varied greatly by region and the trend of a decreasing prevalence with age was more evident in 1990 as compared to 2005, where some regions, e.g. South East Asia showed an exceptional increase with age. Yang CX et al[16] documented the percentages of the chronic HBV carriers with liver histopathology inflammatory grading & fibrosis staging. He observed Significant difference existed among groups in general [P value <0.01]. Yun-Fan Liaw & Maurizia R Brunetto et al[17] documented the differences about geographical variations. In our study majority of the patients were completely asymptomatic. In a similar study conducted by Fattovich et al[18] stated that out of 105 patients of chronic hepatitis B infection; 56 were asymptomatic. However Hoofnager et al[19] pointed out that, symptoms of chronic hepatitis B infection correlate poorly with the activity of liver disease.

In the studies performed by Razario et al[20] and Fattovich et al[18] most of the cases had mild and minimal activity. Fibrosis was assessed for each case in our study. The results were similar with Tor Borg et al[21] who found that on the evaluation of 174 liver biopsies; maximum patients were in stage 0 and I and cirrhosis identified in only 9 [5.2%] cases. Similarly, Huo et al[22] in a study of 1355 patients only found 6 patients of cirrhosis. Sigal SH et al[23] concluded

Table 3: Status of HBeAg-positive and HBe antigen negative [Anti HBe antibody positive] and its comparison with age, sex, symptoms, grade & stage

	HBeAg positive [n = 110]	HBe antigen negative [Anti HBe positive] [n = 85]	Statistical Analysis	P value / Significant
Mean age	33.56 ± 4	43.3 ± 7	t = 12.23 d.f. = 193 [unpaired 't' test]	0.0001 H.S.
Male	90	72	Fisher Exact Test [2 tailed]	0.70 N.S.
Female	20	13		
Symptomatic	21	10	Fisher Exact Test [2 tailed]	0.235 N.S.
Asymptomatic	89	75		
Minimal to mild activity	104	75	Fisher Exact Test [2 tailed]	0.122 N.S.
Moderate to severe activity	6	10		
Stage 0,I,II	92	80	Fisher Exact Test [2 tailed]	0.026 Significant
Advanced fibrosis and cirrhosis [Stage III,IV]	18	5		

[d.f.= Degree of freedom]

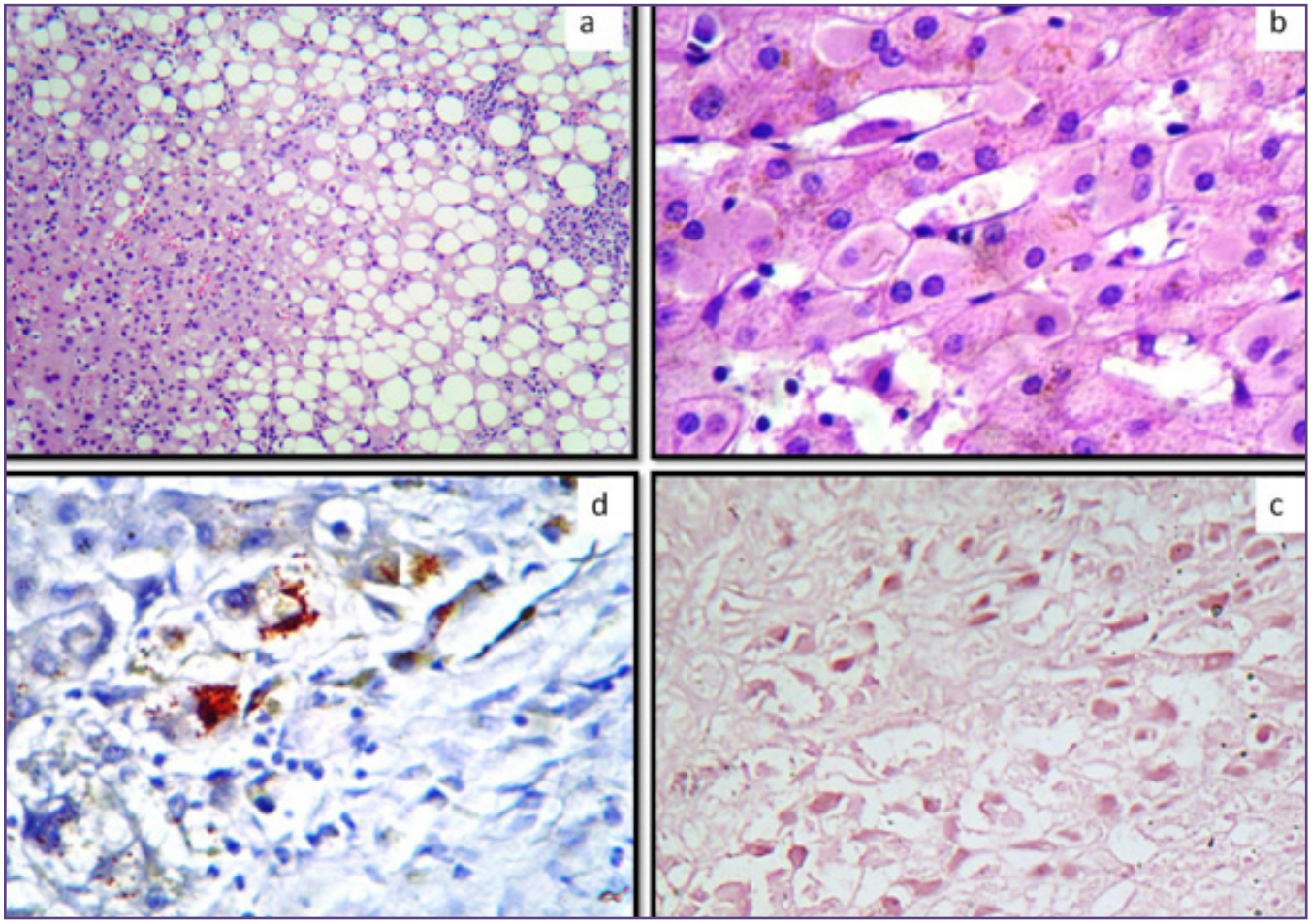

Fig. 3: Ignored area's in the current scoring system [a] Macrovesicular Steatosis with confluent & spotty necrosis [H & E, x 100]. [b & c] Ground glass hepatocytes in a patient with hepatitis B virus infection [H & E, x 400 & Orcein x 400]. [d] Increased copper seen as red granules in the hepatocytes [Rhodanine x 400].

that there was no correlation between inflammatory activity and age, ethnicity, enzyme aminotransferase, bilirubin & HBV DNA levels, HBeAg seropositivity. They stated that patients with high-grade inflammation had greater degrees of hepatic decompensation and high-grade inflammation is common in end-stage HBV cirrhosis, but it is not readily detected by virologic and biochemical parameters. High-grade inflammation is associated with a greater degree of liver decompensation. Our study showed significant differences between age, symptoms and advanced fibrosis in HBeAg positive and HBe antigen negative while grading matches poorly.

Another histological feature frequently encountered in many of our cases was a fatty change. According to Peng et al[24] & Tor Borg F et al[21] steatosis can be due to hepatitis B virus which facilitates development of steatosis and fatty change seen in 54% of cases of hepatitis B. Other histological features which were identified were ductular proliferation, ground glass appearance etc. Yang JD et al[25] & Yang LM et al[26] also concluded in their study that elevated enzyme levels do not mean more disease activity as patients with normal AST and ALT levels were found to have prominent inflammation and fibrosis. We also performed HBeAg and anti-HBe in all the patients and analysed the results. 110 patients were HBeAg positive while 85 patients were positive for anti-HBe antibody. There was no significant difference in AST and ALT levels in both these groups. HBeAg positive chronic hepatitis patients had minimal to mild activity while anti-HBe antibody positive ones had moderate to severe activity and this finding was statistically significant. Similar results were also presented by Zarski et al[27] who also inferred that anti HBe antibody positive patients had more active, advanced and progressive liver disease as compared to patients having HBeAg. However, no difference in enzyme levels was noted. Mc Mohan et al[28] also quoted that in

Alaskan natives with chronic hepatitis B infection, loss of HBeAg was more likely to occur in older patients and presence of anti-HBe antibody is associated with severe necroinflammation and more progression to cirrhosis.

Difficulty were noted in stage 3 and 4 biopsies because by definition Fibrosis with architectural distortion, but no obvious cirrhosis was stage 3 and Probable or definite cirrhosis came under stage 4. But these terms had not been discussed and explained in a meaningful sense. If incomplete septae were found with but with thick septae, what category would it be! Also the HBs Ag positivity, copper binding protein load, Steatosis had not been given any importance in the existing scoring systems.

The histological scores are not representing the measurement of continuous variable but different categories of severity. The inter-observer variation cannot be avoided. Sampling variation may exist on needle biopsy at different parts of liver, in term of both necro-inflammation and fibrosis.

Limitation of this study was related to factors that were not considered in our analysis such as genotype information & data pertaining to secondary causes of steatosis in HBV infection.

Conclusion

Liver biopsy remains the main diagnostic standard test in evaluating the hepatic pathology in hepatitis B infection. Modified HAI scoring system by Ishak and staging system by Scheuer are excellent for evaluating liver biopsy. By grading & staging with this system the physician can plan their management well. Age & enzyme levels had no correlation with disease activity and fibrosis. However, clinical features had a significant correlation with disease activity, particularly advanced fibrosis. Amount of fibrous tissue can be given in reports with Progressive & Regressive fibrotic changes. Cirrhosis should not be considered as an end point rather clinical correlation could be done to document compensated or decompensated cirrhotic changes. Size of nodules, thickness of septa and amount of fibrosis should be included in the revised classification which is the need of future. Thus sub classification is the necessity for clinical usefulness. It does not matter which system we follow for grading & staging. Pathologist & physicians can determine what is most comfortable and what type of reporting makes sense. Give graphic representation and write the system clearly in diagnosis.

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Competing Interests

None Declared

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