Original Article

Histological grading and staging of liver and its relation to viral loads in chronic anti-HBe positive hepatitis

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Received: 13 Jan 2013
Revised: 18 March 2013
Accepted: 3 April 2013

Abstract

Background: Hepatitis activity index (HAI) and fibrosing stage are two important findings during the evaluation of liver samples in patients with chronic hepatitis B. The aim of this study was to determine the HAI and fibrosing stage in patients with anti-HBe positive chronic hepatitis B.

Methods: Liver biopsy slides of 72 patients were evaluated at the Department of Pathology in two teaching hospitals of Babol University of Medical Sciences, Iran, from April 2006 to August 2011. Total HAI or grading as well as its components including piecemeal necrosis, confluent necrosis, spoty necrosis, portal inflammation and fibrosis scores or staging in considering with viral loads more or less than 10^5 copies/ml were enumerated according to Ishak scoring system.

Results: The mean age of these patients was 34.4±12 years. Fifty-six patients had viral load> 10^5 copies/ml. Piecemeal necrosis and grading scores with viral load (10^3,10^3-10^5 and >10^5 copies/ml) were 0.8±0.7, 0.9±0.4, 1.8±1 and 3.8±1.9, 4.4±2, 5.9±2.6, respectively (p=0.005 and p=0.04, respectively). There was not any significant difference with fibrosis stage regarding different viral loads. In total, 18 cases had fibrosis scores > 1 and 24 cases had confluent necrosis. HAI≥4 was seen in 29 (60.4%) of the 48 cases without confluent necrosis and in 23 out of 24 cases with confluent necrosis (p=0.007).

Conclusion: The results show that piecemeal necrosis and higher grading scores are associated with higher viral loads. The presence of confluent necrosis is associated with more severe diseases.

Keywords: Grade, Stage, Confluent necrosis, Viral load.

Caspian J Intern Med 2013; 4(3):681-685

More than 400 million people in the world are suffering from chronic HBV infection and up to 40% of these patients have progressed to cirrhosis and end stage liver disease (1, 2). Hepatitis B virus DNA (HBV DNA) quantification is now playing an important role in the assessment of the liver disease (3-4). A cut-off level of 10^5 copies/ml of serum has been recommended to differentiate between carriers and patients with chronic hepatitis (5, 6).

Chronic hepatitis B is characterized by the grade (the degree of inflammation and hepatocellular injury) which is thought to lead to the fibrosis stage. The end stage of chronic hepatitis is cirrhosis with clinical decompensation (7, 8). Hepatitis activity index (HAI) or grading and fibrosis stage or staging are two important points that determine the mild, moderate or the severity of the disease (9). Several scoring systems have been developed and among them Ishak scoring system is more popularly used (10). Thus, the aim of this study was to determine the HAI stage and fibrosing stage in patients with anti-HBe positive chronic hepatitis B.

Methods

This is a retrospective study in which we analyzed the histological files of liver on 72 anti HBe positive cases who underwent liver biopsy at the Infectious Diseases Research Center of Babol University of Medical Sciences between April 2006 to August 2011.

Hepatitis activity index (HAI) or grading and fibrosis scores or staging were determined according to the scheme given by Ishak et al. (10). At first, a record was provided for each patient and viral loads, ALT levels, hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), antibody against hepatitis B e antigen (anti-HBe), α-fetoprotein were noted on it.The viral markers were tested in Elisa (HBsAg, from Bio Meraux, the Netherlands; anti-HBe, HBeAg from Dia.Pro Diagnostic BioProbes, Italy).

Serum ALT levels were determined by using a Hitachi 704 auto-analyzer, Tokyo, Japan. The upper limit of the normal value introduced by the manufacturer was 40 IU/L for both men and women. Liver biopsy was done on patients who had viral loads $>10^5$ copies /ml with any levels of ALT and those with viral loads$< 10^5$ copies/ml with ALT >40 IU/L in two occasions from 3 months apart. For quantification of HBV DNA, Roter-Geen 3000 (Corbett Research) using Artus HBV RG PCR kit (Qiagen, Humburg, Germany) was used. According to the kit manufacturer's instructions, the sensitivity of the test was 3.8 IU/mL (1 IU=7 copies/ml). The liver biopsy specimens were fixed in 10% formalin and sections were stained with hematoxylineosin, reticulin, and masson's trichrome stains. This study was approved by the Infectious Diseases Research Center, Babol University of Medical Sciences Iran. Informed consent was obtained from each patient for performing liver biopsy.

Statistical analysis: The data were analyzed using SPSS version 15. The relation between grading (HAI) and staging with regard to viral loads $< 10^3$,10^3 - 10^5 and more than 10^5 copies/ml were compared. T-test and Fisher exact tests and Mann-Whitney test were used when appropriate. Anova test and Kruskal-Wallis test were used to compare the severity of liver injury regarding the different viral loads.

Results

Seventy-two (55 males, 17 females) samples were evaluated. The mean age of these patients was 34.4±11.9 years. The mean ALT level was 105±10.5 IU/L. Fifty-six patients had viral load > 10^5 copies/ml. Nine cases (all were males) had viral load < 10^3 and 7 (one female and six males) between 10^3-10^5 copies/ml (p=0.143) (table 1). Eight out of 56 cases with viral loads> 10^5 copies/ml had ALT<40 IU/L. With ALT<40 IU/L, HAI>3 was seen in 5 (62.5%) of 8 cases and in 38 (79.2%) of 48 cases with ALT>40IU/L in subjects with viral loads $>10^5$copies/ml (p=0.37). HAI ≤3 was seen in 5 out of 9 subjects with viral loads <1000, in 2 out of 7 subjects with viral loads 10^3-10^5 and in 13 out of 56 cases viral loads > 10^5 copies/ml (p=0.132). Mean grading and staging of the liver with different viral loads are shown in table 1.

Table 1. Histological findings of liver regarding viral load in 72 subjects with anti-HBe positive chronic hepatitis

Histologic finding (no. of case)	Mean±SD	p-value
Piecemeal necrosis		
VL < 10^3 (9)	0.8±0.7	
VL:10^3-10^5 (7)	0.9±0.4	0.005
VL >10^5 (56)	1.8±1	
Confluent necrosis		
VL < 10^3 (9)	0.8±0.7	
VL:10^3-10^5 (7)	0.3±0.5	0.94
VL >10^5 (56)	0.4±0.5	
Spoty necrosis		
VL < 10^3 (9)	1.3±0.5	
VL:10^3-10^5 (7)	1.6±1	0.2
VL >10^5 (56)	1.8±0.9	
Portal inflammation		
VL < 10^3 (9)	1.3±0.5	
VL:10^3-10^5 (7)	1.7±0.8	
VL >10^5 (56)	2±0.9	0.1
Grading		
VL < 10^3 (9)	3.8±1.9	
VL:10^3-10^5 (7)	4.4±2	0.04
VL >10^5 (56)	5.9±2.6	
Staging		
VL < 10^3 (9)	0.9±0.8	
VL:10^3-10^5 (7)	0.4±0.8	0.2
VL >10^5 (56)	1.2±1.3	

VL; viral load, SD; standard deviation

Viral load $>10^5$ copies/ml were associated with more subset of liver lesion regarding piecemeal necrosis, and grading (table 2). In total, 18 cases had fibrosis scores > 1

and 24 cases had confluent necrosis. HAI≥4 was seen in 29 (60.4%) of the 48 cases without confluent necrosis and in 23 out of 24 cases with confluent necrosis (p=0.007). Overall, 47 (65.3%) cases had fibrosis scores≥1. Total Ishak score ≥3 was seen in 62 (86.11%) subjects. No cirrhosis or hepatocellular carcinoma was detected in our cases. Alpha-fetoprotein in all cases was in the normal ranges.

Table 2. Liver histology regarding viral loads more or less than 10^5 copies/ml in 72 cases of anti- HBe positive chronic hepatitis B

Histologic finding	$< 10^5$ N=16	$>10^5$ N=56	p-value
Piecemal necrosis	0.81±0.5	1.8±1.1	0.001
Confluent necrosis	0.3± 0.5	0.4±0.5	0.75
Spotty necrosis	1.4±0.73	1.8±0.9	0.11
Portal inflammation	1.5±0.6	1.96±0.9	0.06
Grading	4±1.9	5.9±2.6	0.004
Staging	0.7±0.8	1.2±1.3	0.12

Discussion

In this study, we found that mean piecemeal necrosis and grading scores were significantly higher in those who had viral loads more than 10^5 copies/ml (table 2). We also found a positive correlation between HBV DNA levels and the grade of histological inflammation as shown by others (11, 12). Piecemeal necrosis shows a diagnostic criterion in chronic active hepatitis that was seen more in those who had higher viral load (7).

Some experts suggested a cut off of 10^5 copies /ml and this was an arbitrary value (6, 13). Subsequent study showed that taking this level of viremia as the cut off, 45% of anti-HBe positive CHB patients would be misclassified as similar to our finding which was 43.8% (7 out of 16 cases) (3). Manesis et al. suggested that a cut-off value of 30000 copies/ml would be a better cut-off for differentiating between carriers and patients with chronic hepatitis, but even this level was shown to misclassify 30% of patients with HBeAg negative CHB (3, 14).

In this study, we found that 56.3% of 16 cases with viral loads< 10^5 copies/ml had significant hepatic lesions. With this level of viral loads, Brunetto found that 23 (29%) of 79 cases had significant hepatic injury which was higher than what we found in this study (15). But our findings were in accordance with the results of Chu et al. These findings emphasize that no single level of HBV DNA load reliably differentiates between carrier subjects and those with significant liver injuries (16).

In our study, the subjects with viral loads <1000 copies/ml, significant histological disease was seen in 44.4% of 9 cases, but other studies in Iran reported that a considerable proportion of cirrhotic patients (36%) had HBV DNA viral load under 10^3 copies/ml (17). In this study, we found that with viral loads $>10^5$ copies/ml, the severity of liver injury was higher than those with viral loads $<10^5$ copies/ml. Tai et al. also reported higher rates of morbidity and cirrhosis in those with viral loads> 10^5copies/ml (18). Therefore, in spite of the viral load, the severity of liver injury may be related to immune response, viral factors such as HBV genotype and mutations in core promoter and precore regions, and environmental factors such as alcohol consumption as shown by others (6, 19-20). In our study, confluent necrosis was seen in 24 (33%) of our cases. It is well known that the presence of confluent necrosis is a representative of severe activity and suggests the possible clinical implications to be considered (21).

Confluent necrosis of any degree in hepatitis B implies the specific clinical events or conditions that have possibly important clinical correlates requiring attention. The small numbers of our cases with confluent necrosis may be related to the size of the obtained specimens of the liver. We included specimens who had more than 6 portal spaces. Colloredo et al. reported that the ideal sample size should be 2 cm long and 1.4 mm wide with no less than 11 to 15 portal tracts (22-23).

In this study, fibrosis was seen in 18 out of 72 cases and most of them had mild fibrosis. The presence of active inflammation in one-third of our patients shows the driving force leading to fibrosis, and increasing of grade is associated with more fibrosis. Severe activity means confluent necrosis (21, 24). Recently, Fibroscan was introduced for the evaluation of liver injury, but this procedure only is able to determine advanced fibrosis (25). Therefore, liver biopsy remains the gold standard for the evaluation of the HAI and fibrosis. The weakness of this study is the small number of patients with viral load less than 10^5 copies/ml. The size of our samples might be another weakness of this study. In summary, the results show that piecemeal necrosis and higher grading scores are associated with higher viral loads. The presence of confluent necrosis is associated with more severe disease.

Acknowledgments

We would like to thank the personnel of the Infectious Diseases Research Center of Babol University of Medical Sciences for helping and giving the records of all patients and the Department of Pathology of Shahid Beheshti Hospital for providing all the slides of the patients.

Funding: This study was supported by the Infectious Diseases Research Center of Babol Medical University (GR032-1389). For the thesis of Shima Soleimani Amiri.

Ethical approval: There was no need for the ethics committee approval.

Conflict of interests: We have no conflict of interest to declare.

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