

## Evaluation of the need for treatment on 72 subjects with anti-HBe positive chronic hepatitis B

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### Abstract

**Background:** Viral load and alanine aminotransferase (ALT) levels may not be the key points for making a decision in the treatment of anti-HBe positive chronic hepatitis B. The purpose of this study was to assess the histological evaluation of liver to find the need for treatment on 72 patients with anti-HBe positive chronic hepatitis B.

**Methods:** The liver biopsy slides of the 72 patients (56 subjects with viral load  $>10^5$  with any ALT levels, 16 cases with viral load  $<10^5$  copies/ml with abnormal ALT levels) were evaluated at the Department of Pathology, Babol University of Medical Sciences, Iran from April 2006 to August 2011. Ishak Scoring system was used to determine the hepatitis activity index (HAI) and fibrosis score. Those with total score  $>3$  were considered for treatment. Data were collected and analyzed.

**Results:** The mean age of the patients was  $34.4 \pm 12$  years. The mean ALT level was  $105 \pm 10.5$  IU/L. The mean HAI with viral loads more or less than  $>10^5$  was  $5.9 \pm 2.6$  and  $4 \pm 1.9$ , respectively ( $p=0.04$ ). HAI  $>3$  was seen in 9 (56.3%) and in 43 (76.8%) subjects with viral loads  $<10^5$  and  $>10^5$  copies/ml ( $p<0.05$ ). No fibrosis was seen in 25 (34.7%) of these cases. Fibrosis stage regarding viral loads more or less than  $10^5$  copies/ml was equal ( $p=0.12$ ). The need for treatment was seen in 62 (86%) patients.

**Conclusion:** The results show that any viral load values may cause significant injuries that need to treatment. Liver biopsy is indicated in any case of anti-HBe with any viral loads with increased ALT levels.

**Keywords:** Hepatitis activity index, Fibrosis, Treatment, Viral load

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The histological features of chronic hepatitis B virus infection (HBV) ranges from minimal, mild, and moderate to severe chronic hepatitis. Moderate to severe chronic hepatitis B have been shown to progress to cirrhosis and end stage liver disease if left untreated (1, 2). Early identification of patients who need to treatment is the key factor in the management of anti-HBe positive chronic hepatitis B (3, 4).

Recently, molecular biology techniques have shifted the focus of HBV diagnosis from serology and histology to genome detection. HBV DNA quantification is now playing an important role in the assessment of viral activity and response to therapy. A cut-off level of  $10^5$  copies/ml of serum has been recommended to differentiate between the carriers and patients with chronic hepatitis (5, 6).

In using the lower cut-off value, the researchers identified a large number of cases who needed treatment (2, 7). But most investigators defined HBV carriers based on persistently normal alanine aminotransferase (ALT) levels without confirming the absence of the disease on liver biopsy.

The degree of activity, unless particularly severe, is not as important as the stage of fibrosis in the decision on whether or not to pursue treatment.

Since the significant cases of anti-HBe positive patients in our regions are precore mutant and have recommended viral loads for differentiation of carrier state to significant diseases who need to treatment have not been clearly determined, therefore, the purpose of this study was to evaluate the need for treatment on 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 Medical University between April 2006 to August 2011. Hepatitis Activity Index (HAI) (grading) and fibrosis score (staging) was done according to the scheme given by Ishak et al. (8).

At first we assessed the viral loads and ALT levels, hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), antibody against hepatitis B e antigen (anti-HBe),  $\alpha$ -fetoprotein for each patient. The viral markers tested in Elisa were the following: HBsAg, from Bio Meraux, the Netherlands; anti-HBe, HBeAg from Dia.Pro Diagnostic BioProbes, Italy.

Serum ALT levels were determined 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, Humberg, Germany) was used. All samples were obtained by using a 16 G Menghini's aspiration needle under lignocaine local anaesthesia. The liver biopsy specimen was fixed in 10% formalin and sections were stained with haematoxylineosin, reticulin, and masson's trichrome stains. Those with total score  $>3$  was considered for treatment.

**Statistical analysis:** The data were analyzed using SPSS version 15. The relation between HAI and fibrosis stage with regard to ALT levels  $<$  or  $>40$  IU/L as well as viral loads more or less than  $10^5$  copies/ml were compared. T test and Fisher exact tests were used when appropriate. The need for treatment in these cases was determined.

## Results

Seventy-two (55 males, 17 females) cases were evaluated. The mean age of these patients was  $34.4 \pm 11.9$  years. The mean ALT level was  $105 \pm 10.5$  IU/l. Fifty-six patients with viral loads  $>10^5$  copies/ml and 16 subjects had viral loads  $<10^5$  copies/ml. 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  $>40$  IU/l in subjects with viral loads  $>10^5$  copies/ml ( $p=0.4$ ). The subjects with viral loads  $<10^5$  copies/ml (16 subjects), HAI  $>3$  were seen in 9 (56.3%) cases and in those with viral loads  $>10^5$  was 43 (76.8%) ( $p<0.05$ ) (table 1).

The mean HAI scores in 56 subjects with viral loads  $>10^5$  copies/ml was  $5.9 \pm 2.6$  and in 16 subjects with viral loads less than  $10^5$  copies/ml was  $4 \pm 1.9$  ( $p=0.04$ ). The mean Fibrosis scores in 56 subjects with viral loads  $>10^5$  copies/ml was  $1.2 \pm 1.3$  and in 16 subjects with viral loads less than  $10^5$  copies/ml was  $0.7 \pm 0.8$  ( $p=0.12$ ). No fibrosis was seen in 25 (34.7%) of these cases and fibrosis score 1 was seen in 29 (40.3%) of these cases. In total, 47 cases had fibrosis scores  $>1$  and 24 cases had confluent necrosis.

Total Ishak score  $>3$  was seen in 62 (86.11%) subjects. No cirrhosis or hepatocellular carcinoma was detected in our cases. A-fetoprotein was within normal range in all cases.

**Table 1. Mean variables investigated in patients with viral load more or less than  $10^5$  copies/ml**

Variables	Viral load (copies/ml) $>10^5$ N=56	$<10^5$ N=16
Mean HAI score (mean $\pm$ SD)	5.9 $\pm$ 2.6	4 $\pm$ 1.9
Mean staging (mean $\pm$ SD)	1.2 $\pm$ 1.3	0.7 $\pm$ 0.8
HAI $>3$ , no (%)	43 (76.8)	9 (56.3)*
Mean AST level	78 $\pm$ 113	56.3 $\pm$ 11*
Mean ALT level (mean $\pm$ SD)	111.4 $\pm$ 99	86.8 $\pm$ 33.8*
Total Ishak score $>3$ no (%)	50 (89.3)	12 (75)

HAI; Hepatitis activity index, ALT; Alanine aminotransferase, AST; Aspartate aminotransferase, SD; standard deviation

\*The differences in these variables in two groups were significant

## Discussion

The histologic evaluation of the liver has remained an important issue regarding the diagnosis and treatment of

patients with chronic hepatitis B. In this study, we considered liver biopsy on patients with viral load  $>10^5$  copies/ml with any levels of ALT or patients with viral load  $<10^5$  copies/ml with abnormal ALT. With viral load  $>10^5$  copies/ml, the degree of HAI was higher than those with viral loads  $<10^5$  copies/ml, but the need for treatment in these two groups were equal (table 1).

Some experts suggested a viral load cut off of  $10^5$  copies/ml as presence of active disease but subsequent studies showed that significant hepatic injuries had been seen in a large number of cases with viral loads  $<10^5$  copies/ml similar to the findings of our study that was 75% (9,5, 6). Manesis et al. recommended a cut off value of  $3 \times 10^3$  copies/ml for differentiating an active or an inactive disease, but they found that they would not consider about 30% of patients with active disease with this cut off value (9, 7). We found that 75% of 16 cases with viral loads  $<10^5$  copies/ml had significant hepatic lesions.

Thus, in accordance with the results of Chu et al. we found that no single level of HBV DNA load reliably differentiated these two groups (9). Therefore, most clinicians rely on liver biopsy for the confirmation of their clinical diagnosis to initiate antiviral treatment or not (10). ALT levels although show hepatic inflammation but was not a reliable test showing active lesion as we found in significant hepatic injuries in 52.5% subjects with normal ALT and all had viral loads  $>10^5$  copies/ml.

Several studies showed significant histological injuries in their cases with normal ALT levels (11-13). In our study the subjects with viral loads  $<10^5$  copies/ml, a significant histological disease was seen in three quarter of our 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. Although we had not found any cases of cirrhosis with any viral loads, however other studies in Iran reported that a considerable proportion of cirrhotic patients (36%) had HBV DNA viral load under  $10^3$  copies/ml (14).

Other researchers also noted that when histological injury was severe the level of viremia was expected to be low (15-17). In our study, a total 16 cases with viral loads  $<10^5$  copies/ml, a significant hepatic injury that needs treatment was seen in 75% cases.

Other studies showed that 29% of 79 cases with these levels of viral load had significant hepatic injury (18). An interesting findings in our patients with viral loads  $>10^5$

copies/ml had higher ALT levels as well as severity of liver injury as compared to that with viral loads  $<10^5$  copies/ml also liver injuries in both groups were significant that needed treatment.

Tai et al. also reported higher rates of morbidity and cirrhosis in those with viral loads  $>10^5$  who had ALT  $>2$  times the upper limit of normal (19). Chun et al. reported that viral load was not related to aminotransferase (20). Our results indicate that it was difficult to define a single HBV DNA value, which could differentiate between the carriers and significant hepatic lesions in anti-HBe positive patients. Most patients with anti-HBe-positive and HBV-DNA positive chronic hepatitis B have HBV variants with mutations in the precore or core promoter region. In patients with HBV variants, progressive liver damage occurred in parallel with relatively high levels of viremia as we found in our patients (21, 22).

The weakness of this study 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 (23).

The low numbers of cases with viral loads  $<10^5$  copies/ml with abnormal ALT level might be another weakness of our study. The results of our assay and the findings of other researchers showed that liver biopsy continued to give essential information of the liver regarding diagnosis and therapeutic management.

In conclusion, the results show that any viral load values may cause significant injuries that need to treatment. Liver biopsy is indicated in any case of anti-HBe with any viral loads with increased ALT levels.

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**Ethical approval:** There was no need for the Ethics Committee's approval.

**Conflict of interest:** We declare that we have no conflicts of interest.

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