

ORIGINAL ARTICLE

ADVANCED LIVER INJURY IN PATIENTS WITH CHRONIC HEPATITIS B
AND VIRAL LOAD BELOW 2,000 IU/mL

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SUMMARY

Introduction: According to the guidelines, the viral load of 2,000 IU/mL is considered the level to differentiate between inactive carriers and HBeAg(-) chronic hepatitis B patients. Even so, liver damage may be present in patients with lower viral load levels, mainly related to regional variations. This study aims to verify the presence of liver injury in patients with viral load below 2,000 IU/mL. **Methods:** Patients presenting HBsAg(+) for more than six months, Anti-HBe(+)/HBeAg(-), viral load below 2,000 IU/mL and serum ALT levels less than twice the upper limit of normality underwent liver biopsy. Clinical and laboratory characteristics were evaluated in relation to the degree of histologic alteration. Liver injury was considered advanced when $F \geq 2$ and/or $A \geq 2$ by the METAVIR classification. **Results:** 11/27 (40.7%) patients had advanced liver injury, with a mean viral load of 701.0 (\pm 653.7) IU/mL versus 482.8 (\pm 580.0) IU/mL in patients with mild injury. The comparison between the mean values of the two groups did not find a statistical difference ($p = 0.37$). The average of serum aminotransferases was not able to differentiate light liver injury from advanced injury. **Conclusions:** In this study, one evaluation of viral load did not exclude the presence of advanced liver damage. Pathologic assessment is an important tool to diagnose advanced liver damage and should be performed in patients with a low viral load to indicate early antiviral treatment.

KEYWORDS: Hepatitis B; Viral Load; Liver injury.

INTRODUCTION

Hepatitis B is one of the most prevalent infectious diseases in the world. According to World Health Organization (WHO), there are 400 million people chronically infected with hepatitis B virus (HBV) worldwide^{1,2}. In Brazil, there are over 75,000 new cases per year and the number of HBsAg-positive people is about 2 million individuals, heterogeneously distributed, with an increase from Southern to Northern regions^{3,4}.

Three stages of chronic HBV infection are recognized: immune tolerance, characterized by the positivity of HBsAg and HBeAg, high levels of viral load (VL), normal alanine aminotransferase (ALT) and no evidence of active liver disease; immunoactive phase or chronic hepatitis B (HBeAg positive, high levels of HBV DNA, elevated ALT, and evidence of active liver disease); HBV inactive carrier or asymptomatic (HbsAg positive without HBeAg, HBV-DNA < 2,000 IU mL, normal ALT). The immunoactive phase is divided into two major forms: HBeAg positive disease (wild-type HBV) and HBeAg-negative disease (variant pre-core, core promoter variant HBV)⁵.

The distinction between inactive hepatitis B and chronic hepatitis B HBeAg negative may be difficult, so that patients may be misdiagnosed and remain at risk of developing cirrhosis or hepatocellular carcinoma (HCC)⁵⁻⁷. Therefore, detection of viral replication, and subsequent quantitation subsidizes the diagnosis, and therapeutic decision and monitoring, as well. Since 2005, it is known that patients with normal ALT could have advanced fibrosis and HCC depending only on VL¹². This information has led to a review of global guidelines and modified the cut-off levels of the viral load to define the diagnosis and the need of treatment to 2,000 IU/mL⁹.

Regarding hepatitis B, fibrosis is the main histological prognostic factor¹³. Many noninvasive methods to evaluate fibrosis have been studied. It is possible to use hematological, biochemical and imaging exams to determine the prognosis^{5,13}. However, these tests have not been validated for routine clinical assessment of intermediate lesions, so that histology remains the gold standard¹³.

Recent studies indicate that, even with viral load of HBV below

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20,000 IU/mL, or even below 2,000 IU/mL, a high degree of liver injury may exist in most patients^{14,15,16,17}. Clinical characteristics and regional variations interfere in the clinical presentation of chronic HBV hepatitis.

The aim of this study was to assess the degree of liver injury in chronic hepatitis B patients with viral load below 2,000 IU/mL and whether there is correlation between clinical and laboratory factors.

METHODS

This is an observational descriptive cross-sectional study based on records and primary data collection from patients with chronic hepatitis B virus, monitored in the Service of Hepatology, University Hospital, Federal University of *Sergipe*, Brazil, from 2006 to 2011.

All the medical records of patients with hepatitis B were surveyed. The patients presenting positive HBsAg for more than 6 months, Anti-HBe(+)/HBeAg(-), serum aminotransaminases (AST/ALT) less than twice the upper limit of normality (ULN), viral load below 2,000 IU/mL and age between 18 and 65 years were selected. Exclusion criteria were: coinfection with HCV and/or HIV, undetectable viral load by PCR; alcoholism (> 20 g/day for women, > 40 g/day for men); clinical acute hepatitis; use of immunosuppressive or hepatotoxic drugs and contraindication to perform a liver biopsy (platelets < 50,000/mm³, INR > 1.7). Patients who agreed with the procedure signed an informed consent and underwent a percutaneous liver biopsy within 6 months from the date of the viral load measurement.

The following data were evaluated: gender, age, ALT, AST, HBV-VL and histopathological changes. The latter were reviewed by an experienced pathologist (H.L.F.B.) who made the descriptions according to the METAVIR classification (A-inflammatory activity, F-Fibrosis)¹³.

To evaluate the liver damage severity, patients were divided into two groups: G1 = patients with mild hepatic injury (A < 2 and F < 2); and G2 = patients with advanced liver injury (A ≥ 2 and/or F ≥ 2).

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 17.0 for Windows, using the Chi-square test, the Student t test and the Pearson correlation. The significance level was set at *p* < 0.05.

The study was approved by the research ethics committee of the Federal University of *Sergipe*, under the protocol CAAE-0038.0.107.000-10 and followed the precepts of the Declaration of Helsinki and the resolution 466/2012 of the National Health Council.

RESULTS

Twenty-seven patients HBsAg(+), anti-HBeAg(+)/HBeAg(-) and VL below 2,000 IU/mL were selected. Of these, 13 (48.1%) were male. The mean age was 36.4 ± 9.2 years (22-59) (Table 1). Fifteen (55.5%) patients reported mild alcohol consumption (< 20 g/day for women and < 40 g/day for men) or sporadic consumption, while 12 (44.5%) were abstainers (Table 1).

Following the METAVIR classification, when the patients were divided according to the severity of liver injury (mild-G1 or

Table 1
Demographic, clinical and laboratory features of 27 patients with chronic hepatitis B HBeAg negative and viral load below 2,000 IU/mL

	Mean ± standard deviation (minimum and maximum)	No. of patients/total%
Age (years)	36.4 ± 9.2 (22-59)	
Gender, male		13/27 (48.1%)
Ethanol ^a		15/27 (55.5%)
Total Bilirubin (n=26)	0.84 ± 0.99 (0.5-5.5)	
Direct Bilirubin (n=24)	0.25 ± 0.78 (0.05-3.9)	
AST* (U/mL)		
Female	24.6 ± 5.9 (17-39)	
Male	37.5 ± 11.0 (22-60)	
ALT [#] (U/mL)		
Female	44.2 ± 9.2 (37-73)	
Male	54.4 ± 13.6 (39-77)	
AST greater than ULN [†]		
Female		0/14
Male		4/13 (30.8%)
ALT > ULN		
Female		0/14
Male		3/13 (23.1%)
Viral Load (IU/mL)	572.1 ± 608.7 (26-1,960)	

^aConsumption of ethanol less than 40g/day for men and 20g/day for women; ULN - upper limit of normality. * The mean difference in AST for men and women was statistically significant (*p* < 0.01); # The mean difference in ALT for men and women was statistically significant (*p* = 0.03); † The difference in proportions of men and women that presented AST values above the normal limit was statistically significant (*p* = 0.02).

advanced-G2), 59.3% (16/27) had mild and 40.7% (11/27) had advanced disease. Seventeen (63.0%) patients were A1 and 10 (37.0%) were A2. Regarding fibrosis, seven (25.9%) were classified as F0, 11 (40.7%) were F1; six (22.2%) were F2, one (3.7%) was F3 and two (7.4%) were F4 (Table 2). The group of patients with advanced liver injury had mean VL of 701.0 IU/mL (SD ± 653.7) versus 482.8 IU/mL (SD ± 580.0) in patients with mild injury (*p* = 0.37). No statistical differences were found with respect to clinical and laboratory variables. Among patients with mild alcohol consumption, 10 (66.7%) had mild hepatic injury and five (33.3%) had advanced lesions, and the comparison of these two groups did not find statistical differences (*p* = 0.3) (Table 3).

Twenty-two (81.5%) patients showed no serum aminotransferases increments. AST was below the upper limit of normality in 85.2% (23/27) of patients, with a mean value of 30.8 ± 10.8 (18-60) U/mL. This level was between one and two times the normal values in four (14.8%) males. No women showed AST above the upper limit of normality. The comparison between mean AST and genders showed a statistically significant difference (*p* < 0.01) (Table 1).

ALT fell below the upper limit of normality in 24 (88.9%) patients, the mean value was 49.11 ± 12.44 (36-77) U/ml and was between one

Table 2

Histological characteristics of 27 patients with chronic hepatitis B HBeAg negative and viral load below 2000 IU/mL

Histologic Findings	No. of patients / total%
METAVIR	
A1	17/27 (63.0%)
A2	10/27 (27.0%)
F0	7/27 (25.9%)
F1	11/27 (40.7%)
F2	6/27 (22.2%)
F3	1/27 (3.7%)
F4	2/27 (7.4%)
Other Histologic Findings	
Ground-glass	8/27 (29.6%)
Steatosis	14/27 (51.9%)
Light	11/14 (78.6%)
Moderate	3/14 (21.4%)
Macro	14/14 (100%)
Micro	0/14
Granuloma ^a	4/27 (14.8%)
Perisinusoidal Fibrosis	0/27
Mallory Hyaline	0/27
Siderosis	0/27

^aSchistosomal granuloma;

Table 3

Relationship between advanced liver injury and demographic, clinical and laboratory characteristics of 27 patients with chronic hepatitis B HBeAg negative and viral load below 2000 IU/mL

	Advanced Liver Injury ^a		P
	Absent	Present	
Age	34.7 ± 9.2	38.8 ± 8.9	0.26
Gender, male	8 (50.0%)	5 (45.5%)	0.82
Ethanol ^b	10 (66.7%)	5 (33.3%)	0.38
Viral load	482.8 ± 580.0	701 ± 653.7	0.37
AST	30.8 ± 11.3	30.9 ± 10.6	0.98
ALT	50.4 ± 14.5	47.1 ± 8.9	0.51
Total Bilirubin	0.65 ± 0.3	1.15 ± 1.55	0.22
Direct bilirubin	0.09 ± 0.03	0.47 ± 1.21	0.25
Total	16 (59.3%)	11 (40.7%)	

^aAdvanced liver injury = fibrosis ≥ 2 and/or Activity ≥ 2 by METAVIR classification; ^b Consumption of ethanol less than 40g/day for men and 20g/day for women.

and two times the normal values in three (11.1%) male patients and, as for the AST, no woman had values above the upper limit of normality. The comparison between mean ALT and genders showed a statistically significant difference ($p = 0.03$) (Table 1).

HBV-VL ranged from 26.0 IU/mL to 1,960.0 IU/mL with a mean of 572.1 ± 608.7 IU/mL (Table 1). Patients were divided into two categories: 10 (37%) patients were between the lower detection limit and 200 IU/mL,

and 17 (63%) were between 201 and 2,000 IU/mL. There was no statistical difference between the VL and the severity of liver injury. Patients with VL between 201 and 2,000 IU/mL had a mean AST level of 33.9 (SD ± 11.4; limits of 17 and 39) U/mL versus 25.6 (± 7.4 SD; limits 19 and 60) U/mL in the group with VL below 200 IU/mL ($p = 0.05$). All the patients with AST/ALT above the normal limits were in the group with higher VL. There were no significant correlations between HBV-VL and the clinical or other laboratory variables (Table 4).

Regarding the histological macroscopic examination, except for one patient, all the others had two or more liver fragments analyzed. The smaller sample had eight portal tracts. Five patients had between 8 and 10 analyzed portal tracts, the others had 11 or more, except for two patients with cirrhosis whose structural changes did not allow the samples to be scored.

Histological analysis revealed the presence of ground-glass appearance in eight (29.6%) patients, and steatosis in 14 (mild in 11 and moderate in three) (51.9%), all of them with a macrogoticular type. Perisinusoidal fibrosis, Mallory hyaline, and siderosis were not found in any of the patients. Schistosomal granuloma was seen in four patients. Of these, three (75%) were in the mild hepatic lesion group and one (25%) in the group of advanced liver injury.

DISCUSSION

The importance of this manuscript is the study of clinical, laboratory and histological features of patients with hepatitis B and VL below 2,000 IU/mL, as well as the significance of the pathological examination to differentiate patients with advanced liver injury from the ones in whom anti-viral treatment is indicated. This is an important issue since there are large geographical variations in the presentation of chronic hepatitis B and the results presented herewith may contribute to add some information to the few studies available in the literature, especially in developing countries.

Eleven (40.7%) patients had advanced liver injury, of whom two (7.4%) had liver cirrhosis, calling attention to the fact that in HBeAg(-) patients with normal ALT levels, a single analysis of VL below 2,000 IU/mL did not exclude the possibility of liver damage, and this is an important indication of anti-viral treatment to prevent progression to the chronic forms of disease. Corroborating our findings, Papatheodoridis *et al.* (2008)¹¹ found moderate or severe inflammatory activity and moderate or severe fibrosis in 14.3% (6/42) and 47% (20/42) of patients with VL less than 2,000 IU/mL, respectively. Kim *et al.* (2010) have also shown similar results regarding the liver damage in patients with low viral load (< 2,000 copies/mL)¹⁸.

Considering the current Protocol Clinical Guideline for the Treatment of Chronic Viral Hepatitis B from the Health Ministry of Brazil²⁰, 22 (81.5%) patients in this study should be monitored for a year to set the stage of chronic hepatitis B. Nine patients (40.9%), who already had advanced liver injury initially, would not have been identified. According to the Brazilian protocol, patients with VL less than 2,000 IU/mL and normal ALT should be followed in a reference service to monitor the transaminases and VL quarterly, for one year²⁰. At the best scenario, patients would have a delay of 12 months until the beginning of the anti-viral treatment.

Table 4

Demographic, clinical, laboratory and histological features of patients with viral load between the lower limit of detection and 200 IU/mL and between 201 and 2000 IU/mL (n = 27)

	HBV DNA		P
	Between the lower detection limit and 200 IU / mL	Between 201 and 2000 IU / mL	
Age	36.9 ± 8.5	36.12 ± 9.77	0.83
Gender, male	4 (30.8%)	9 (69.2%)	0.51
Ethanol ^a	5 (33.3%)	10 (66.7%)	0.65
AST*	25.6 ± 7.4	33.94 ± 11.4	0.05*
ALT	45.7 ± 11.7	51.12 ± 12.7	0.28
Total Bilirubin	0.5 ± 0.15	1.01 ± 1.19	0.21
Direct Bilirubin	0.75 ± 0.02	0.36 ± 0.98	0.39
METAVIR ^b			0.95
Light Injury	6 (37.5%)	10 (62.5%)	
Advanced Injury	4 (36.4%)	7 (63.5%)	
Histological Changes other not METAVIR ^c			
Steatosis	6 (42.9%)	8 (57.1%)	0.51
Frosted glass	1 (12.5%)	7 (87.5%)	0.08
Granuloma ^d	2 (50%)	2 (50%)	0.56
Total	10 (37%)	17 (63%)	

^aConsumption of ethanol less than 40g/day for men and 20g/day for women. ^bLight liver Injury = F < 2 and A < 2; Advanced Liver Injury = F ≥ 2 and/or A ≥ 2; ^cSiderosis, Mallory hyaline and perisinusoidal fibrosis were not found in any patient; ^dSchistosomal granuloma; * The mean difference in AST was statistically significant (p = 0.05) between the group with viral load between the lower detection limit and 200 IU/mL and the group with viral load from 201 to 2000 IU/mL.

In contrast, in Germany²⁴, histology is the main determinant to start the treatment. Therefore, liver biopsy is indicated for all the patients at the time of diagnosis. The Asian Pacific Association for the Study of the Liver (APASL) claims that the liver biopsy is a good assessment in a higher number of situations^{9,25}.

Serum levels of liver enzymes and VL (less than 200 IU/mL, or 201.0 - 2,000 IU/mL) were not able to differentiate patients with or without advanced liver injury.

Schistosomiasis is an endemic disease in our region, therefore, the finding of schistosomal granulomas in the liver biopsy is not uncommon^{26,27}. This finding was present in samples of four patients and could be a confounding factor in the analysis of the lesion stage, but three (75%) of these patients were in the group without liver damage, minimizing the impact of schistosomiasis in the degree of liver fibrosis in the patients of this study.

Steatosis was found in 51.9% of patients with chronic hepatitis B. Studies have shown a higher prevalence of steatosis in hepatitis B compared to the general population^{13,28}. The incidence observed in this study is in accordance with data described by other authors²⁴.

The limitations of this study were that it was an observational cross-sectional study that assessed only one VL measure. However, when the reality of each geographical area is considered, it was observed that

due to difficulties in accessing health services, many patients fail to perform follow-up examinations as frequently as they should, affecting the quality of provided care. The small sample of this study may also be a limitation. Nevertheless, an important amount of patients with hepatic lesions has been detected.

In conclusion, considering that none of the clinical factors and laboratory parameters was able to differentiate patients with and without advanced liver damage, it is suggested that liver biopsy should be indicated in patients with chronic hepatitis B independently of the VL level, in order not to delay the antiviral treatment.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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