

ORIGINAL
ARTICLECorrelation between Hepatitis B Viral DNA Load
and Extent of Liver Pathology in Patients with
Chronic Hepatitis B

Shahinul Alam*, Nooruddin Ahmad, Khorshed Alam, Golam Mustafa, Mobin Khan

Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka, Bangladesh

Background and Aims: Hepatitis B virus (HBV) DNA level is used as a criterion for antiviral therapy in patients with chronic hepatitis B (CHB). However, the relationship between serum HBV-DNA level with liver histology remains controversial. The objective of this study was to determine the relationship between HBV-DNA load with liver histopathology in patients with CHB.

Methods: The study was conducted between October 2003 and May 2007 in the Department of Hepatology of Bangabandhu Sheikh Mujib Medical University. 209 consecutive patients with CHB were studied. The liver histology was graded by Knodell's criteria.

Results: 175 (83.5%) of patients were male; the patients had a mean $\pm$ SD age of 26.6 $\pm$ 8.4 years, had a mean $\pm$ SD HBV DNA level of 6.9 $\pm$ 1.6 log₁₀ copies/mL, necro-inflammatory score of 6.8 $\pm$ 3.2 and fibrosis score of 1.7 $\pm$ 1.2. Eight-one (38.8%) patients were HBeAg-negative. There was no correlation between the HBV-DNA load and either of necro-inflammatory activity or fibrosis. Eight (9.9%) patients in HBeAg negative group and 2 (1.6%) in HBeAg positive group had DNA level below the recommended level of treatment but had significant pathology.

Conclusions: HBV-DNA load does not have any correlation with the histological abnormalities and fibrosis in liver and the lowest level of HBV-DNA to start the treatment requires reconsideration.

Keywords: Hepatitis B Virus, Hepatitis, Liver Fibrosis, Histological Activity, DNA, Chronic hepatitis B

Introduction

Hepatitis B virus (HBV) infection is a global public health problem. Chronic hepatitis B (CHB) is caused by persistent infection with HBV-a unique DNA virus that replicates through an RNA intermediate produced from a stable covalently closed circular DNA molecule⁽¹⁾. It is estimated that there are at least 400 million HBV carriers in the world and that up to one million die annually due to hepatitis B-associated liver disease⁽²⁾. HBV infection predisposes patients to serious sequelae such as liver cirrhosis and hepatocellular carcinoma. Patients with significant hepatic inflammation and fibrosis are at the highest risk of these complications⁽³⁻⁷⁾. Bangladesh with a population of 150 million has a hepatitis B surface antigen (HBsAg) seroprevalence of 7.2%-7.5% in healthy adult population^(7, 8). Determinants of clinical outcome of CHB still remain unknown.

Seroconversion from hepatitis B e-antigen (HBeAg) to hepatitis B e-antibody (anti-HBe) was previously believed to be accompanied by cessation of HBV replication and remission of the liver disease^(9, 10). Currently, measuring HBV-DNA level becomes the most important serological marker to study the natural history of CHB and to assess the treatment efficacy. With the availability of sensitive HBV-DNA assays, most patients who were previously

* Correspondence:

Shahinul Alam, M.D., Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka, Bangladesh.

Tel: +880 291 30102

Fax: +880 2 81 11069

E-mail: shahinul67@yahoo.com

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considered to have non-replicate infection are found to have detectable serum HBV-DNA; HBV replication persists throughout the course of chronic HBV infection.

In recent years, HBV-DNA level exceeding 10^5 copies/mL in HBeAg-positive patients and 10^4 copies/mL in HBeAg-negative patients have become an important marker for determining the need for treatment⁽¹¹⁾. Although the serum HBV-DNA level is used as a criterion for antiviral therapy in patients with CHB, the relationship between serum HBV-DNA level and liver pathology remains controversial. The primary objective of the present study was to determine whether there is any relationship between HBV-DNA level with the degree of necro-inflammation and fibrosis in both HBeAg-positive and negative patients. Furthermore, we studied whether patients with HBV-DNA levels below 10^5 copies/mL in HBeAg-positive patients and below 10^4 copies/mL in HBeAg-negative patients showed necro-inflammatory activity and fibrosis.

Materials and Methods

Patients were included in this study if they had 1) a positive HBsAg test for at least six months, 2) positive or negative HBeAg, 3) and a positive HBV-DNA of any amount. Those with other causes of hepatitis, prior to antiviral treatment, with overt cirrhosis or liver cancer were excluded from the study. The protocol was reviewed and approved by the Department Ethical Review Committee. The study was conducted between October 2003 and May 2007 in the Department of Hepatology, Bangabandhu Sheikh Mujib Medical University (BSMMU) Hospital Dhaka, Bangladesh. During this period, 209 consecutive patients who fulfilled the inclusion criteria underwent liver biopsy. Before liver biopsy informed written consent was taken from every patient. The liver histology was assessed by a pathologist who was blind to the result of the liver function test results and HBV-DNA levels.

The liver histology was graded by the histologic activity index (HAI) according to the criteria of Knodell, *et al.*⁽¹²⁾. The total HAI score comprises of two major components namely necro-inflammation and fibrosis which includes "piecemeal necrosis," "lobular necrosis" and "inflammation, portal inflammation and fibrosis." HBV serological markers were detected using enzyme-linked immunosorbent assays (ELISA, Abbott Laboratories, Chicago, IL, USA). Serum HBV-DNA was determined by target-amplification assay

based on competitive polymerase chain reaction (Amplicor HBV Monitor™, Roche Molecular Systems, Pleasanton, CA) with a detection range of 300 to 10^6 copies/mL that could be increased with dilution. ALT, HBsAg, HBeAg, anti-HBe (ELISA), and Anti-HCV (DiaSorin, Italy) were measured for all patients.

Results were expressed as mean±SD or percentage. HBV-DNA level and ALT did not have a normal distribution, thus, they were analyzed after logarithmic transformation. Independent-sample *Student's t*-test and one-way ANOVA were used to compare mean of continuous variables. χ^2 test was used to compare categorical data. Pearson's correlation coefficient was calculated for measuring of correlations. A $P < 0.05$ was considered statistically significant. Statistical analysis was performed by SPSS® 11.5 for Windows®.

Results

Baseline characteristics

One-hundred and seventy five (83.7%) of 209 studied patients were male. The patients had a mean±SD age of 26.6±8.4 (range: 14-55) years, HBV-DNA level of 6.9±1.6 log₁₀ copies/mL, necro-inflammatory activity score of 6.8±3.2 (range: 1-14) and fibrosis score of 1.7±1.2 (range: 0-4). Eighty-one (38.8%) patients were HBeAg-negative. HBeAg-negative patients were older ($P < 0.005$) than those positive for HBeAg. HBeAg-negative patients had similar ALT levels ($P = 0.408$) and necro-inflammatory score ($P = 0.662$) to HBeAg-positive patients. However, the former group had higher fibrosis score ($P < 0.05$), lower DNA levels ($P < 0.005$) and a higher frequency of cirrhosis ($P < 0.05$) (Table 1).

Table 1. Baseline characteristics of patients with chronic hepatitis B.

Characteristics	HBeAg-positive	HBeAg-negative	P value
n	128 (61.2%)	81 (38.8%)	
Age (yrs)	24.1±7.2	30.1±8.7	<0.005
Male (%)	84.5	62.2	-
Mean±SD serum HBV-DNA (Log ₁₀ copies/mL)	7.6±1.3	5.8±1.4	<0.005
Mean±SD histological grade	6.7±2.9	6.9±3.6	0.662
Mean±SD histological stage	1.4±1.1	1.9±1.3	<0.05
Cirrhosis	2	9	<0.005
Mean±SD ALT activity (U/L)	100.6±169.1	83.7±84.9	0.408

Relationship between HBV-DNA level and liver histology

There was no correlation between the HBV-DNA level and necro-inflammatory score in the whole study group ($r=0.186$), HBeAg-positive ($r=0.094$) and HBeAg-negative ($r=0.789$) patients (Figure 1). Fibrosis was not also correlated to the viral DNA load in either of the groups ($r=0.481$, $r=0.869$, and $r=0.084$, respectively) (Figure 2). If we classified the patients into four groups with DNA levels of $<10^5$, 10^5 - $<10^7$, 10^7 - $<10^9$, and $\geq 10^9$ copies/mL, no significant difference in neither histological activities nor ALT levels among the groups were observed (Table 2).

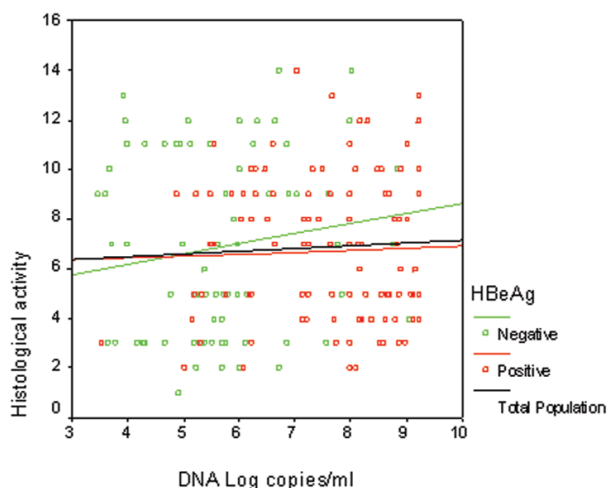


Figure 1. Correlation between HBV-DNA and histological activity.

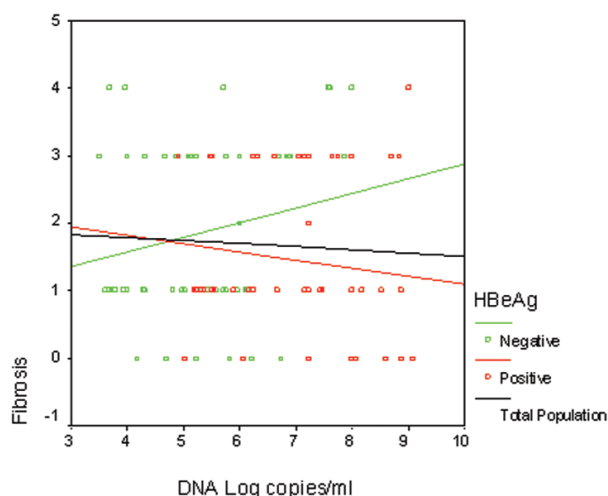


Figure 2. Correlation between HBV-DNA load and fibrosis score.

Table 2. Necro-inflammatory activity and ALT stratified by DNA level.

DNA load (copies/mL)	$<10^5$ (n=230)	10^5 - $<10^7$ (n=76)	10^7 - $<10^9$ (n=93)	$\geq 10^9$ (n=17)
Mean $\pm$ SD Necro-inflammatory activity	7.2 $\pm$ 3.8	6.5 $\pm$ 3.2	6.7 $\pm$ 3.0	7.9 $\pm$ 3.5
Mean $\pm$ SD ALT activity (U/L)	73.8 $\pm$ 86.7	102.2 $\pm$ 215.0	88.6 $\pm$ 65.7	112.2 $\pm$ 90.7

What should be the recommended DNA level for treatment?

Ten patients in this series had DNA levels below the recommended DNA level of treatment and had significant liver pathology; the extent of pathology was not significantly ($P=0.31$) different in those with the DNA levels below and above the recommended level. This was more important in HBeAg-negative patients because eight (9.9%) had DNA levels $<10^4$ copies/mL with a mean $\pm$ SD necro-inflammatory score of 8.3 ± 3.7 while two (1.6%) HBeAg-positive patients had DNA levels $<10^5$ copies/mL with a significant necro-inflammatory activity.

Discussion

In this study, the HBV-DNA level was not correlated to the necro-inflammatory activity of HBeAg-positive and -negative patients with CHB. Fibrosis was also not correlated with any of the groups. Furthermore, in our study, no difference in the histological score was found with respect to DNA levels, regardless of the status of HBeAg, suggesting that no correlation exists between HBV-DNA level and liver histology in either HBeAg-positive or -negative patients. Correlation between HBV-DNA level and the grade and stage of CHB remains controversial because there were contradictory results from different studies (13-18). Our results contradicted those of Korean studies (14, 15), who showed an inverse correlation between the virus DNA load and histological activity in HBeAg-positive patients. This difference may be attributed to the older age of our study population.

No correlation was reported between the DNA load and histological activity in most of studies on HBeAg-positive patients with CHB (13, 16, 19-22). We also found similar results. Several studies revealed a positive correlation between the virus DNA load and histological activity in HBeAg-negative patients (13, 15, 23). However, in some studies, as we did, no correlation was found between the DNA load and histological activities in HBeAg-

negative patients with CHB (16, 19). Our results showed that the HBV-DNA level could not reflect the extent of liver damage which may be due to the fact that HBV itself is not directly cytopathic but the extent of liver injury depends on the host immune response (24). For those patients in the immunotolerant phase, although the HBV-DNA level may reflect a high viral replication, the immune-mediated attack might have not yet been started. During the immunoclearance phase, there will also be a lack of correlation between the HBV-DNA level and the severity of liver pathology because a markedly enhanced immune response may lead to a very low viremic levels even though the ALT level remains very high (13, 25, 26).

The lowest DNA level detected in this series was 3000 copies/mL. We found that 9.9% of HBeAg-negative and 1.6% of HBeAg-positive patients with CHB who had significant histological activities had DNA levels below the recommended level for treatment. Similar results were reported when chronic hepatitis, cirrhosis and hepatocellular carcinoma were also found with lower HBV-DNA level (11). Here, the DNA level would lead to misclassification of patients with CHB and possibly denial of treatment, especially in those with HBeAg-negative CHB. The limitation of the present study was that we estimated the HBV-DNA level only at one time-the time of biopsy. Whereas the HBeAg-negative patients have gotten an erratic pattern of ALT changes and the HBV-DNA level can have frequent fluctuations (27, 28).

Conclusions

The HBV-DNA load had no correlation with the extent of liver pathology and the lowest level of HBV-DNA to start the treatment requires further reconsideration.

References

1. Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok AS. Management of hepatitis B: summary of a clinical research workshop. *Hepatology* 2007; **45**: 1056-75.
2. Conjeevaram HS, Lok AS. Management of chronic hepatitis B. *J Hepatol* 2003; **38**: S90-103.
3. Fattovich G, Brollo L, Giustina G, et al. Natural history and prognostic factors for chronic hepatitis type B. *Gut* 1991; **32**: 294-8.
4. Liaw YF, Tai DI, Chu CM, Chen TJ. The development of cirrhosis in patients with chronic type B hepatitis: a prospective study. *Hepatology* 1988; **8**: 493-6.
5. Yu MW, Hsu FC, Sheen IS, et al. Prospective study of hepatocellular carcinoma and liver cirrhosis in asymptomatic chronic hepatitis B virus carriers. *Am J Epidemiol* 1997; **145**: 1039-47.
6. Weissberg JI, Andres LL, Smith CI, et al. Survival in chronic hepatitis B. An analysis of 379 patients. *Ann Intern Med* 1984; **101**: 613-6.
7. Islam MN, Islam KM, Islam N. Hepatitis-B virus infection in Dhaka, Bangladesh. *Bangladesh Med Res Counc Bull* 1984; **10**: 1-6.
8. Khan M, Ahmad N. Seroepidemiology of HBV and HCV in Bangladesh. *International Hepatology Communications* 1996; **5**: 744-8.
9. Hoofnagle JH, Dusheiko GM, Seeff LB, Jones EA, Waggoner JG, Bales ZB. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. *Ann Intern Med* 1981; **94**: 744-8.
10. Realdi G, Alberti A, Rugge M, et al. Seroconversion from hepatitis B e antigen to anti-HBe in chronic hepatitis B virus infection. *Gastroenterology* 1980; **79**: 195-9.
11. Lok AS, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; **45**: 507-39.
12. Knodell RG, Ishak KG, Black WC, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; **1**: 431-5.
13. Yuen MF, Ng IO, Fan ST, et al. Significance of HBV DNA levels in liver histology of HBeAg and Anti-HBe positive patients with chronic hepatitis B. *Am J Gastroenterol* 2004; **99**: 2032-7.
14. Cho SC, Lee SH, Shinn JJ, et al. HBV DNA levels, aminotransferase and histological activity in young male patients with HBeAg positive chronic hepatitis B (in Chinese). *Taehan Kan Hakhoe Chi* 2002; **8**: 44-51.
15. Kim TH, Kim YS, Yeom JJ, et al. Relevancy between liver injury, serum HBV-DNA, and intrahepatic HBeAg in young male chronic HBV carriers (in Korean). *Korean J Gastroenterol* 2004; **44**: 84-92.
16. Manesis EK, Papaioannou GV, Sevastianos V, Cholongitas E, Papaioannou C, Hadziyannis SJ. Significance of hepatitis B viremia levels determined by a quantitative polymerase chain reaction assay in patients with hepatitis B e antigen-negative chronic hepatitis B virus infection. *Am J Gastroenterol* 2003; **98**: 2261-7.
17. Chan HL, Tsang SW, Liew CT, et al. Viral genotype and hepatitis B virus DNA levels are correlated with histological liver damage in HBeAg-negative chronic hepatitis B virus infection. *Am J Gastroenterol* 2002; **97**: 406-12.
18. Peng J, Luo K, Zhu Y, Guo Y, Zhang L, Hou J. Clinical and histological characteristics of chronic hepatitis B with negative hepatitis B e-antigen. *Chin Med J* 2003; **116**: 1312-7.
19. Shao J, Wei L, Wang H, et al. Relationship between hepatitis B virus DNA levels and liver histology in patients with chronic hepatitis B. *World J Gastroenterol* 2007; **13**: 2104-7.
20. Sakhuja P, Malhotra V, Gondal R, Sarin SK, Guptan R, Thakur V. Histological spectrum of chronic hepatitis in precore mutants and wild-type hepatitis B virus infection. *Trop Doct* 2004; **34**: 147-9.
21. Lindh M, Horal P, Dhillon AP, Norkrans G. Hepatitis B virus DNA levels, precore mutations, genotypes and histological activity in chronic hepatitis B. *J Viral Hepat* 2000; **7**: 258-67.
22. Zavaglia C, Mondazzi L, Maggi G, et al. Are alanine aminotransferase, hepatitis B virus DNA or IgM antibody to hepatitis B core antigen serum levels predictors of

- histological grading in chronic hepatitis B? *Liver* 1997; **17**: 83-7.
23. Nabuco LC, Villela-Nogueira CA, Perez RM, *et al.* HBV-DNA levels in HBsAg-positive blood donors and its relationship with liver histology. *J Clin Gastroenterol* 2007; **41**: 194-8.
 24. Yim HJ, Lok AS. Natural history of chronic hepatitis B virus infection: what we knew in 1981 and what we know in 2005. *Hepatology* 2006; **43**: S173-81.
 25. Dusheiko G, Paterson A. Hepatitis B core and surface antigen expression in HBeAg and HBV DNA positive chronic hepatitis B: correlation with clinical and histological parameters. *Liver* 1987; **7**: 228-32.
 26. Cahen DL, van Leeuwen DJ, ten Kate FJ, Blok AP, Oosting J, Chamuleau RA. Do serum ALT values reflect the inflammatory activity in the liver of patients with chronic viral hepatitis? *Liver* 1996; **16**: 105-9.
 27. Chu CJ, Hussain M, Lok AS. Quantitative serum HBV DNA levels during different stages of chronic hepatitis B infection. *Hepatology* 2002; **36**: 1408-15.
 28. Mommeja-Marin H, Mondou E, Blum MR, Rousseau F. Serum HBV DNA as a marker of efficacy during therapy for chronic HBV infection: analysis and review of the literature. *Hepatology* 2003; **37**: 1309-19.