

RAPID COMMUNICATION

Relationship between hepatitis B virus DNA levels and liver histology in patients with chronic hepatitis B

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Abstract

AIM: To study the relationship between hepatitis B virus (HBV) DNA levels and liver histology in patients with chronic hepatitis B (CHB) and to determine the prevalence and characteristics of hepatitis B e antigen (HBeAg) negative patients.

METHODS: A total of 213 patients with CHB were studied, and serum HBV DNA levels were measured by the COBAS Amplicor HBV Monitor test. All patients were divided into two groups according to the HBeAg status. The correlation between serum HBV DNA levels and liver damage (liver histology and biochemistry) was explored.

RESULTS: Of the 213 patients with serum HBV DNA levels higher than 10^5 copies/mL, 178 (83.6%) were HBeAg positive, 35 (16.4%) were HBeAg negative. The serum HBV DNA levels were not correlated to the age, history of CHB, histological grade and stage of liver disease in either HBeAg negative or HBeAg positive patients. There was no correlation between serum levels of HBV DNA and alanine aminotransferase (ALT), aspartate aminotransferase (AST) in HBeAg positive patients. In HBeAg negative patients, there was no correlation between serum levels of HBV DNA and ALT, while serum DNA levels correlated with ALT ($r = 0.351$, $P = 0.042$). The grade (G) of liver disease correlated with ALT and AST ($P < 0.05$, $r = 0.205$, 0.327 respectively) in HBeAg positive patients. In HBeAg negative patients, correlations were shown between ALT, AST and the G ($P < 0.01$, and $r = 0.862$, 0.802 respectively). HBeAg negative patients were older (35 ± 9 years *vs* 30 ± 9 years, $P < 0.05$) and had a longer history of HBV infection (8 ± 4 years *vs* 6 ± 4 years, $P < 0.05$) and a lower HBV DNA level than HBeAg positive patients (8.4 ± 1.7 Log HBV DNA *vs* 9.8 ± 1.3 Log HBV DNA, $P < 0.001$). There were no significant differences in sex ratio, ALT and AST levels and liver histology between the two groups.

CONCLUSION: Serum HBV DNA level is not correlated to histological grade or stage of liver disease in CHB patients with HBV DNA more than 10^5 copies/mL. Compared to HBeAg positive patients, HBeAg negative patients are older and have a lower HBV DNA level and a longer HBV infection history. There is no significant difference in sex ratio, ALT and AST levels and liver histology between the two groups.

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Key words: Hepatitis B virus; Hepatitis B virus DNA; Chronic hepatitis B; Liver histology

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INTRODUCTION

Hepatitis B virus (HBV) infection is a significant problem in the world. It is estimated that over 350 million persons have chronic hepatitis B (CHB) and more than one million individuals die of HBV-related chronic liver disease annually^[1]. China is a high endemic area of HBV infection, where the prevalence of CHB is 9.09%^[2]. Chronic HBV infection still represents a serious health problem in China.

Availability of sensitive HBV DNA assays, knowledge of the HBV genome organization and replication cycle, and understanding of the host immune response to HBV infection have changed our concept of the natural history of chronic HBV infection^[3]. Most patients who were previously considered to have non-replicative infection have detectable serum HBV DNA and HBV replication persists throughout the course of chronic HBV infection. In addition, chronic hepatitis is characterized by negative hepatitis B e antigen (HBeAg), detectable HBV DNA, elevated aminotransferase, and continuous necroinflammation^[4].

Serum HBV DNA level is used as a criterion for antiviral treatment in patients with CHB. Nevertheless, the relationship between serum HBV DNA level and liver histology remains controversial. The primary aim of this study was to investigate whether serum HBV DNA level

is related with liver histology. The results of our study demonstrate that serum HBV DNA level has a relatively high sensitivity and a wide detection range^[5].

MATERIALS AND METHODS

This was a retrospective study testing stored sera from Chinese patients in Hepatic Department of People's Hospital, Peking University. According to the recommendations of American Association for the Study of Liver Disease for monitoring patients chronically infected with HBV, liver biopsy was performed in HBeAg positive patients with elevated ALT levels and HBV DNA greater than 10^5 copies/mL^[6]. Liver biopsy was performed in 213 patients with HBV DNA greater than 10^5 copies/mL to evaluate liver damage from September 2001 to July 2005. All patients gave their written consent before liver biopsy. None of them received interferon or antiviral therapy before liver biopsy.

All patients had positive HBsAg for more than 6 months. Patients co-infected with hepatitis C virus and hepatitis D virus accompanying autoimmune hepatitis, primary biliary cirrhosis, alcoholic hepatitis, drug hepatitis and Wilson's disease were excluded.

Serological HBV DNA assay

All the sera were collected one week before liver biopsies and frozen at -20°C till HBV DNA testing. ALT and AST were detected by the Hitachi 7600 Series automatic biochemical analyzer (Hitachi, Tokyo, Japan). HBsAg, HBeAg, anti-HBe, anti-hepatitis C virus (third generation assay) and anti-hepatitis D virus were tested with enzyme-linked immunosorbent assay kits (Abbott Laboratories, Abbott Park, IL, USA).

Quantification of HBV DNA was performed according to the manufacturer's instructions by the Cobas Amplicor HBV Monitor test (Roche Diagnostic Systems, Branchburg, NJ, USA). The detection of HBV DNA ranged $300\text{--}10^6$ copies/mL. To extend this range, samples with high HBV DNA levels were retested after dilution.

Liver histology assessment

Sections of the liver biopsy specimens were stained with hematoxylin-eosin and Masson, and assessed by a pathologist unaware of the clinical and virological results. The evaluation of liver pathology followed the modified criteria for grading and staging of chronic hepatitis. The degree of necro-inflammation was classified into G0-4 and fibrosis was staged as S0-4^[7].

Statistical analysis

Statistical analyses were carried out with the Statistical Program for Social Sciences. Categorical data were tested by Chi-square test. Student *t* test was used for comparison of parametric quantitative data and the Mann-Whitney or Kruskal-Wallis test for similar comparison of nonparametric data. Relations between two quantitative variables were performed by Spearman's correlation analysis. Two tailed *P* value of less than 0.05 was considered statistically significant. Results are presented as mean \pm SD.

Table 1 HBeAg negative correlation coefficient and HBeAg positive correlation coefficient

Patients characteristic	HBeAg correlation coefficient	Negative <i>P</i>	HBeAg correlation coefficient	Positive <i>P</i>
Age (yr)	-0.194	0.271	0.016	0.828
History (yr)	-0.295	0.090	-0.072	0.342
Serum ALT	0.351	0.042	0.018	0.811
Serum AST	0.334	0.054	0.039	0.603
Histological grade	0.265	0.130	-0.035	0.641
Histological stage	-0.108	0.544	0.139	0.064

RESULTS

Characteristics of the patients

A total of 173 males and 40 females were enrolled in the study. Their mean age was 31 ± 9 years, ranging from 16 to 65 years. All patients had elevated ALT levels and the median level was 131 U/L (range 48-411 U/L). Serum HBV DNA levels were higher more than 10^5 copies/mL. The median Log HBV DNA level was 9.66 copies/mL (range = 6.0-14.7) in 10 patients. According to the criteria for chronic hepatitis, the median (range) grading and staging scores were 2 (0-4) and 2 (0-4), respectively. Of the 213 patients, 178 (83.6%) were HBeAg positive, 35 (16.4%) were HBeAg negative.

Correlation of serum HBV DNA level to demographic data, liver histology and laboratory findings

HBV DNA level correlated with the age, history of CHB in either HBeAg negative or HBeAg positive patients. In both HBeAg negative and HBeAg positive patients with CHB, HBV DNA was not related to histological grade and stage of liver disease (Table 1). There was no correlation between the levels of HBV DNA, ALT and AST in HBeAg positive patients (*P* = 0.811 and 0.603, respectively). In HBeAg negative patients, there was no correlation between the levels of HBV DNA and AST (*P* = 0.054), while serum HBV DNA level was correlated to ALT (*P* < 0.05 = 0.042), and the correlation coefficient was 0.351 (Table 1).

Patients were divided into four groups (HBV DNA level $\leq 10^7$ copies/mL, $\leq 10^9$ copies/mL, $\leq 10^{11}$ copies/mL, $\leq 10^{13}$ copies/mL). In terms of histological grade and stage, patients with their HBV DNA level $\leq 10^{13}$ copies/mL did not differ from those with their BV DNA level $\leq 10^7$ copies/mL (Table 2).

Relationship between liver histology, ALT and AST

There was a correlation between the grade and stage of liver disease and ALT, AST (*P* < 0.01, *r* = 0.744 and 0.741 respectively) in HBeAg positive patients. However, there was no correlation between ALT, AST and the stage of liver disease in HBeAg negative patients. The correlation between the grade of liver disease and ALT, AST was higher than that between the stage of liver disease and ALT, AST in either HBeAg positive or HBeAg negative patients.

Comparison HBeAg negative with HBeAg positive patients

HBeAg negative patients were older and had a longer

Table 2 Histological scores of CHB patients with respect to different HBV DNA levels

	≤ 10 ⁷ copies/mL (n = 14)	HBV DNA 10 ⁷ copies/mL < HBV DNA level ≤ 10 ⁹ copies/mL (n = 31)	Levels 10 ⁹ copies/mL < HBV DNA level ≤ 10 ¹¹ copies/mL (n = 145)	10 ¹¹ copies/mL < HBV DNA level ≤ 10 ¹³ copies/mL (n = 23)	P
Grades					
Median score (range)	2 (1-3)	2 (1-3)	2 (0-4)	2 (1-3)	0.995
Stages					
Median score (range)	2 (0-4)	2 (0-4)	2 (0-4)	2 (0-4)	0.187

history of HBV infection and a lower HBV DNA level than HBeAg positive patients ($P < 0.05$). There was no significant difference in sex ratio, ALT and AST levels and liver histology between the two groups. The clinical and laboratory data of 213 patients are shown in Table 3.

DISCUSSION

All participants had elevated HBV DNA level greater than 10⁵ copies/mL detected by a highly sensitive PCR assay with the detection limit of 300 copies/mL for serum HBV DNA level, which suggested that HBV replication was still active in both HBeAg positive and negative CHB patients. It was reported that HBV replication persists throughout the whole course of chronic HBV infection^[3]. Moreover, elevated HBV DNA level is a strong risk factor for hepatocellular carcinoma independent of HBeAg, serum ALT level, and liver cirrhosis^[8].

In our study, HBV DNA level was not correlated with liver histology between HBeAg positive and negative patients with CHB. However, whether HBV DNA level is correlated with liver histology in HBeAg negative patients remains controversial because different methods and assays were used in different studies^[9-12] and HBeAg negative patients had different HBV DNA levels. Furthermore, in our study, no different histological scores were found with respect to HBV DNA levels, whatever the status of hepatitis B e antigen was, suggesting that no correlation exists between HBV DNA levels and liver histology in either HBeAg positive or negative patients with HBV DNA levels more than 10⁵ copies/mL.

We did not find the correlation between HBV DNA levels and ALT, AST in HBeAg positive patients. In HBeAg negative patients, serum HBV DNA level was not correlated with AST, but with ALT. However, the correlation coefficient was not high. Our results show that HBV DNA level could not reflect liver damage, as far as CHB patients with HBV DNA higher than 10⁵ copies/mL were concerned. It is known that HBV itself is not directly cytopathic and host immune response plays a pivotal role in HBV-related liver diseases^[3]. Application of sophisticated immunological techniques has demonstrated that patients with chronic HBV infection have impaired immune response to HBV^[13], which may explain why HBV DNA level is not an indicator for liver damage.

Table 3 Comparison of demographic, clinical and virological data between HBeAg negative and positive patients mean ± SD

	HBeAg-positive patients	HBeAg-negative patients	P
Male: Female	136:31	37:9	0.245
Age	30 ± 9	35 ± 9	0.011
History	6 ± 4	8 ± 4	0.036
ALT (U/L)	151 ± 82	162 ± 94	0.501
AST (U/L)	98 ± 65	113 ± 103	0.418
Log 10 HBV DNA	9.8 ± 1.3	8.4 ± 1.7	0.000

In the present study, 16.4% patients fulfilled the definition of HBeAg negative chronic hepatitis B. It was reported that the prevalence of CHB is lower than 33.9% and 35.9% in Chinese individuals^[12,14], which is, however, consistent with the prevalence in Hong Kong population^[11]. The difference may be due to the following factors. First, patients in our study were younger than those in the study of Li *et al*^[14]. As we know, sero-conversion from HBeAg to anti-HBe occurs in 4%-10% of patients during the immune clearance stage. After 5 to 10 years of follow-up, 50% and 70% of HBeAg positive persons still undergo HBeAg sero-conversion^[6]. Second. All patients never received anti-HBV therapies such as interferon or nucleoside analogue treatment. Since all patients had elevated ALT, our sample was biased to patients with active chronic hepatitis B. In addition, geographical variation in HBV genotypes in study population would influence the prevalence of CHB. Finally, ALT levels would fluctuate and HBeAg status of HBV would change as CHB progresses in some HBeAg negative patients. The prevalence of CHB increases in HBeAg negative persons and decreases in HBeAg positive persons, which may be due to the awareness of HBeAg negative CHB, decrease in new HBV infection, and aging of existing carries^[3].

In our study, HBeAg negative patients were older and had a longer HBV infection history than HBeAg positive patients, which is consistent with other studies^[9-11]. HBV infection usually occurs at birth or during early childhood in Asia. The longer the HBV infection is, the more spontaneous the immune clearance occurs, thus leading to HBeAg sero-conversion and HBV replication inhibition. At the same time, immune pressure may also cause pre-core or basic core promoter mutations, which may explain why HBeAg negative patients are older and have a longer HBV history than HBeAg positive ones^[11,15]. In our study, although HBeAg positive patients had a higher HBV DNA level, HBV DNA level was as high as 10¹² copies/mL in HBeAg negative patients, indicating that there is still active HBV replication in HBeAg negative patients. Additionally, there were no significant differences in sex ratio, ALT and AST levels and liver histology between HBeAg positive and negative patients, showing that HBeAg negative hepatitis may be at a late phase in the natural history of chronic HBV infection, rather than a result of *de novo* infection with a mutated variant^[16].

So far liver histology is still considered to be the gold standard for assessing chronic hepatitis. In this study, higher serum ALT and AST levels were not associated

with liver histological grades, and the correlation between liver histological grade and ALT, AST was higher than that between the stage of liver disease and ALT, AST in both HBeAg positive and negative patients, suggesting that ALT and AST levels could reflect the inflammatory activity in the liver of patients with CHB.

In conclusion, HBV DNA level is not correlated with liver histology in CHB patients. HBeAg negative patients have different clinical features. Considering the fluctuation of serum HBV DNA in CHB patients, a longer and closer monitor is needed for patients with CHB infection.

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