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HBV DNA Viral Load and Chronic Hepatitis Infection in Different Stages

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Background and Aims: **The significance of hepatitis B virus (HBV) serum titers has been examined in several clinical situations. Although most studies addressing this question have shown a correlation between virus serum titer and severity of liver damage, some others have failed to show any correlation between them. The aim of the present study was to determine any correlation between HBV viral load and the severity of liver disease.**

Methods: **200 patients, including inactive carriers (n=104), patients with chronic active hepatitis (n=74: 55 HBeAg negative and 19 HBeAg positive) and with cirrhosis (n=22) entered the study. Quantitative serum HBV RNA assay was carried out using the PCR Amplicore technique.**

Results: **The mean age of the patients was 37 ± 12 years. A significant correlation was observed between HBV DNA viral load and ALT in cirrhotic patients (p=0.035, r=0.451). No correlation was observed between serum HBV DNA viral load and the histopathological score or grade, ALT or AST level in other CLD types (p>0.05).**

Conclusions: **Our results indicate that the severity of liver disease is independent of serum levels of hepatitis B virus. The correlation between serum titers of hepatitis B virus and severity of liver disease clearly require further investigation.**

Keywords: **Hepatitis B, HBV DNA viral load, Liver damage, Aminotransferase**

Introduction

Viral hepatitis B is still a major health problem in many parts of the world. Hepatitis B virus (HBV) has been reported to be the most prevalent cause of cirrhosis and chronic hepatitis in Iran and some other developing countries [(1)]. It has also been reported that 65% of all chronic hepatitis B patients in Iran are HBeAg-neg [(2)]. Patients infected with HBV have different disease stages, which accompanies with varying degrees of liver damage. Assessment of disease activity over time is of great importance in the management of chronic HBV infection.

The evaluation of patients with HBV infection has evolved from serological to molecular diagnostic assays. Within the molecular assays, the new highly sensitive techniques of quantification of serum HBV DNA titer have improved our understanding of the pathogenesis and natural history of HBV infection and facilitated the monitoring of response to treatment [(3, 4)]. The National Institutes of Health (NIH) Workshop on "Management of Hepatitis B" held in September 2000, proposed HBV DNA level of 10^5 copies/ml for differentiation of chronic hepatitis B from the inactive carrier state [(5)]. Several studies have assessed the correlation between serum HBV viral load and severity of liver damage, as judged by means of clinical and laboratory parameters [(6-13)]. The profile of correlation between HBV viral load and severity of liver damage seems to be different for the case of HBeAg-pos and HBeAg-neg. For the case of HBeAg-pos, almost all studies have shown no correlation between HBV DNA titers and liver damage [(6, 14)]; however, two recent studies reported that patients with less liver damage had higher viral load [(5, 15)]. For the case of HBeAg-neg, most studies have shown that patients with less liver damage have lower viral load[(10,12-14)]; however,

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some others have failed to observe such an association[4,7]. Despite the controversy in the results of studies assessing the association between DNA viral load and liver damage, NIH has not yet revised its previous recommendation about the cutoff point, and this is still used widely all around the world.

In this study, we aimed to determine if there is any correlation between HBV viral load and the severity of liver disease in inactive carriers, HBeAg-pos and HBeAg-neg patients with chronic active hepatitis and cirrhosis.

Materials and methods

Patients

200 patients with chronic hepatitis B infection, diagnosed on the basis of a positive HBsAg longer than 6 months, enrolled into our study. All patients were negative for other causes of chronic liver diseases including hepatitis C and D infections. None of the patients were treated with antiviral drugs such as interferon-alpha and nucleoside analogues. The stage of the liver disease was determined with respect to AASLD practice guidelines for chronic hepatitis B [16]. Informed consent was received from all subjects.

Detection of biochemical parameters

The serum samples for determination of biochemical parameters, HBV DNA levels, and serological markers were collected simultaneously at the time of registration. Common biochemical parameters relevant to liver inflammatory activities and function including ALT, AST, Alk-P, total serum protein, albumin, and bilirubin were determined by Hitachi 7170 automatic biochemistry analysis system (Japan) in all patients.

Histological investigation

All patients suspicious of having chronic active hepatitis, who had not undergone a liver biopsy within past two years, were asked to undergo a liver biopsy, with respect to the AASLD practice guidelines for chronic hepatitis [16]. Histological grade and score of liver was determined according to the severity of inflammation and fibrosis based on Ishak Scoring [17].

Quantitative HBV analysis

Quantitative assay of HBV levels was performed by the Cobas Amplicore HBV monitor test (Somerville, New Jersey USA) technique which has a lower limit detection of 200 virus mEq/ml [5] in Keyvan laboratory.

Data analysis

Statistical analysis was carried out using SPSS® software for Windows® 10. The values of serum viral titers were presented as mean ± standard deviation (SD). Data of serum viral titers and liver tests were analyzed by means of three distinct statistical ways. At first, serum viral titers and liver tests were both considered as a quantitative variable equal to the raw titer. In this way, linear regression was performed for assessing the correlation between serum viral titer and liver tests. Liver tests were then considered as qualitative variables on the basis of the cut-off point (AST/ALT≤43 versus AST/ALT>43). In this way, Kruskal-Wallis test was used to evaluate any difference in HBV viral load in patients with normal or abnormal liver enzymes. Finally, we considered both serum viral titers and liver tests as qualitative variables. In order to evaluate any correlation between them, Fisher exact test was used. P values less than 0.05 were considered as significant.

Results

104 carriers (52%), 74 patients with chronic active hepatitis (37%) and 22 patients with cirrhosis (11%) entered our study. Of the 200 patients, from 12 to 72 years (mean 37 ± 12 years), 157 (78.5%) were male and 43 (21.5%) were female.

HBV titer, stage and HBe Ag status

There was a significant difference in serum HBV DNA viral load between inactive carriers (9148 ± 15984), HBeAg negative chronic active hepatitis patients (7976669 ± 23385970), HBeAg positive patients (46107368 ± 59635000) and cirrhotic patients (5688464 ± 16108504) (P=0.001 Kruskal-Wallis test).

Liver tests

We did not find any linear correlation between serum viral titers and AST/ALT (P>0.05 - Spearman correlation test) in any stage of the disease, but cirrhotic patients had a significant correlation between HBV DNA viral load and ALT (p=0.035, r=0.451 Spearman's correlation) (Chart 1).

The HBV DNA level was not different in patients with normal or abnormal AST/ ALT (P>0.05 - Man

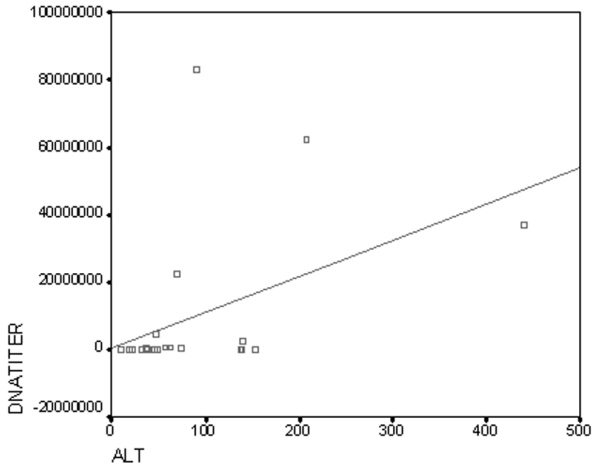

Chart 1. Correlation between HBV DNA viral load and ALT in cirrhotic patients) p=0.035, r=0.451- Spearman's test).

Whitney test) in inactive carrier HBeAg-pos and HBeAg-neg patients with chronic active hepatitis.

There was also not any significant difference in frequency of serum viral loads>10^5 copies/ml between patients with normal and abnormal ALT/ AST (P>0.05 - Fisher Exact test) in any stage of the disease (Table 1).

Histopathological findings

Liver biopsy was done for 49 subjects (9 carriers, 38 chronic active hepatitis patients and 2 cirrhotic ones).

Histopathological total score

There was no correlation between HBV DNA levels and histopathological total score of liver in any stage of the disease (P>0.05 - Spearman test).

Histopathological Grade

There was not any significant difference in the mean serum viral load between patients with mild to moderate (0-6) and patients with severe (7-12) grades of liver histopathology in any stage of the disease (P>0.05 - Man Whitney test).

There was not any significant difference in relative frequency of HBV DNA level≥10^5 between patients with mild to moderate (0-6) and those with severe (7-12) grades of liver histopathology in any disease stage (P>0.05 - Fisher Exact test).

Histopathological stage

There was not any significant difference in mean serum viral load between patients with mild (0-2) and patients with moderate to severe (3-6) stages of liver histopathology in any stage of the disease (P>0.05 - Man Whitney test).

We did not find any significant difference in relative frequency of HBV DNA level≥10^5 between patients with mild (0-2) and patients with moderate to severe (3-6) stages of liver histopathology in any stage of the disease (P>0.05 - Fisher Exact test).

Table 1. correlation between HBV viral load and the severity of liver disease in inactive carriers, HBeAg-pos & HBeAg-neg patients with chronic active hepatitis and cirrhosis.

		N	HBV DNA (copies/ml)	P value (1)	HBV DNA >10^5	P value (2)
Inactive carrier						
	ALT ≤ 41 U/L	104	9148± 15984		0(0%)	
	ALT> 41 U/L	-	-		-	
Chronic active hepatitis HBeAg(+)						
	ALT ≤41 U/L	5	56974000 ± 78675986	p>0.05	5 (100%)	p>0.05
	ALT > 41 U/L	14	42226428 ± 54386842		14 (100%)	
Chronic active hepatitis HBeAg(-)						
	ALT ≤ 41 U/L	12	53801 ± 16991436	p>0.05	10 (83.3%)	p>0.05
	ALT> 41 U/L	43	8701283 ± 25001673		39 (90.7%)	
Cirrhosis						
	ALT ≤41 U/L	6	78882 ± 17907	p>0.05	1 (16.7%)	p>0.05
	ALT> 41 U/L	16	13356971 ± 25587639		9 (56.3%)	
	AST ≤ 41 U/L	8	728287 ± 1582420	p>0.05	3 (37.5%)	p>0.05
	AST> 41 U/L	14	14882752 ± 27107940		7 (50%)	

1) Man Whitney test

2) Fisher Exact test

Discussion

The aim of this study was to determine if there was any correlation between HBV viral load and the severity of liver disease in different stages of chronic hepatitis B and different status of the HBeAg.

In our study, HBV DNA viral load was higher in HBeAg-pos chronic active hepatitis patients than in those with HBeAg-neg, and HBV DNA viral load was higher in both than in cirrhotic patients. Xie et al. also reported that HBV DNA levels in HBeAg-pos group were significantly higher than those in HBeAg-neg group[7]. Our result indicates that a considerable proportion of cirrhotic patients (36%) have HBV DNA viral load under 10^3 copies/ml. While fewer hepatocytes are present in cirrhosis, a lower viremia level may be present despite the high intrahepatocyte viral load.

In our study, we did not observe any correlation between HBV DNA viral load and liver damage (liver enzyme or histopathological findings) in any disease stage, except in cirrhotic patients, in whom a positive correlation was seen between viral load and ALT (chart 1).

Our results showed that HBV DNA level is varied widely in patients with chronic active hepatitis. Although the highest viral load was up to 19.7×10^7 copies/ml in the group of chronic active hepatitis in our study, 8.1 % of these subjects were detected to have an HBV viral load less than 10^5. The threshold HBV DNA level associated with progressive liver disease is not known and may be dependent on host factors such as immune response, viral factors such as HBV genotype and mutations in core promoter and precore regions, and environmental factors such as alcohol consumption[5]. It is known that in the immunotolerance phase of the HBV infection, viral load remains high up to 10-30 years, which may confirm our results. The pathogenesis of hepatic damage is believed to be immune-mediated hepatic injury induced by HBV, in which cytokines may play a mediation role in chronic HBV liver injury. Many patients with chronic HBV infection also have fluctuating HBV DNA levels [5], which may be the cause of this finding. However, the exact cause clearly requires further investigation.

Xie et al. failed to show any significant correlation between HBV DNA viral load and ALT in HBeAg-neg chronic hepatitis patients[7]. Chun et al. reported that viral load was not related to aminotransferase[18]. Habersetzer et al. showed that in patients with chronic hepatitis B-eAg positive, HBV DNA, (detected by PCR) was not correlated with ALT value [19].

Conclusion

It seems that determining an absolute cut-off point for viral load can not be achieved based on just few studies, and has been previously recommended to be reconsidered by Chu and Lok and Manesis et al. [5, 8]. Yuen et al. have also recommended that a better cut-off HBV DNA level is needed to be defined; as in their study, they established 4.3% of the patients with HBV DNA level<10^5 had fibrosis[6]. Manesis et al. reported that 30,000 copies/ml is more useful in differentiation between HBeAg-neg chronic hepatitis B and inactive hepatitis B surface antigen carrier state [8].

In conclusion, our findings indicate that the severity of liver disease is independent of serum levels of hepatitis B virus. These findings are important since they have a direct impact on the current debate regarding the determination of HBV≥10^5 as a cut-off point for selection of patients who need treatment, or for differentiation of chronic hepatitis from inactive carriers. A lower cut off point is recommended for HBeAg-neg chronic hepatitis B and cirrhotic patients. Consideration of liver enzymes, pathology and viral load all together are recommended to select the patients who need treatment. The association between HBV viral load and ALT in cirrhotic patients highlights the value of antiviral therapy in cirrhotic patients with higher ALT levels, in centers with no access to check HBV DNA titer. Therefore, if ALT level is high in cirrhotic patients, consider it as high viral load and start antiviral therapy.

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