RESEARCH ARTICLE

Alanine aminotransferase is more sensitive to the decrease in hepatitis B virus-DNA load than other liver markers in chronic hepatitis B patients

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Background: A direct correlation between hepatitis B virus DNA (HBV-DNA) and liver markers has not been identified in chronic hepatitis B (CHB) patients. However, the effect of HBV-DNA changes on liver markers remains unclear. We explored the association between decreased HBV-DNA and liver markers in CHB patients.

Methods: Chronic hepatitis B patients who visited Jinhua Central Hospital twice were selected for analysis. Finally, 171 participants with a 1-log reduction in HBV-DNA between the two visits were enrolled as the case group, and 158 participants with no significant changes in HBV-DNA were enrolled as the control group.

Results: There was no significant correlation between HBV-DNA and liver markers ($P > .05$). However, in longitudinal analysis, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transpeptidase (GGT) were significantly different between the two tests ($P < .05$) in the case group. Conversely, there was no significant difference in the control group. When HBV-DNA decreased >26 times, ALT was reduced by half or more. A similar trend was observed with a decrease of >63 times for AST and a decrease of >76 times for GGT.

Conclusions: A large change in HBV-DNA can lead to a significant variation in liver markers. In particular, ALT was more sensitive than other liver markers to a reduction in HBV-DNA.

KEYWORDS

CHB patients, HBV-DNA load, liver markers

1 | INTRODUCTION

Hepatitis B virus DNA (HBV-DNA) load is recognized as a direct and reliable marker of HBV infection, and it can reflect the strength of HBV replication and infectivity.^{1,2} HBV infection is a major factor that causes hepatitis, liver cirrhosis, and liver cancer. The immune response plays an important role in HBV-related liver injury.^{3,4} Total protein, albumin, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase, etc. are the most commonly used liver markers to evaluate liver status.⁵

Several studies^{5,6} have reported that there is no direct relationship between HBV-DNA load and liver markers, and HBV-DNA load and ALT were found to be weakly correlated ($r = .234$, $P < .05$).⁵ Those studies^{5,6} performed cross-sectional analysis and demonstrated that liver markers do not always change with changes in HBV-DNA load, but none of them performed longitudinal analysis and reported the association between changes in HBV-DNA load and liver markers. We aimed to explore the effect of the decreased HBV-DNA load on liver markers in individuals with chronic hepatitis B (CHB).

2 | METHODS

2.1 | Population

From June 2015 to October 2016, 400 CHB patients who subsequently visited (interval between the two visits: 1-3 months) Jinhua Central Hospital at least twice were randomly selected. We collected two test results of these CHB patients, including data on HBV-DNA load and liver function. Then, we compared these two results; participants who had 10 times lower HBV-DNA load in the second visit than that in the first visit were selected as the case group, and participants who were similar to the case group but had no significant changes (<10 times) in HBV-DNA load were selected as the control group. Among the participants, those who had a history of excessive drinking, liver cancer, heart diseases, and muscle injury were excluded. Finally, 171 participants and 158 participants were enrolled as the case group and control group, respectively, in this study; 65.4% were males, aged from 13 to 69 with an average age of 38.27 ± 12.62 years. The interval of time between first and second visit of the participants to the hospital was 1-3 months with an average interval 58.51 ± 12.73 days. All of the participants took antiviral medications.

2.2 | Measurements

All blood samples were tested in the Department of Clinical Laboratory, Jinhua Central Hospital. Liver markers were assessed by Beckman Coulter Automatic Biochemical Analyzer using manufacturer-provided reagents. HBV-DNA load was tested by a Roche PCR quantometer, and the reagents were provided by Shenzhen Piji Biochemistry Company. All experimental procedures were carried out according to protocol.

2.3 | Statistical analysis

Normal distribution data are expressed as mean $\pm$ SD. Pearson correlation was applied to analyze the cross-sectional relationship between

HBV-DNA load and liver markers. A paired t-test was used to compare the difference in liver makers between the two results of the participants. ROC curve was applied to evaluate the decrease in HBV-DNA load that could induce a reduction in more than half in liver markers. We classified markers in the second test that decreased half or more compared with those in the first test as the state variable and classified the changes in HBV-DNA load (results of the second test/results of the first test) as the test variable. HBV-DNA load was a highly skewed variable; thus, logarithmic transformation was performed for analysis. All analyses were performed using SPSS 19 (IBM, Armonk, NY, USA), and $P < .05$ was considered as statistically significant.

3 | RESULTS

The correlation between HBV-DNA load and liver markers is displayed in Table 1. There was a weak correlation between HBV-DNA load and albumin ($r = .13$, $P = .031$). However, no statistically significant correlation was found between HBV-DNA load and other liver markers ($P > .05$). There was also no statistically significant correlation between age and ALT ($r = .012$, $P = .907$).

Table 2 shows the comparison between the liver markers of the first test and the second test. In the first test, there was no significant difference between the liver markers in the case group and those in the control group ($P > .05$). In the case group, statistical differences between the two tests were found for ALT (140.28 ± 38.02 vs 41.63 ± 15.97 , $P = .02$), AST (127.74 ± 34.63 vs 37.48 ± 11.21 , $P = .01$), and GGT (54.17 ± 20.66 vs 34.95 ± 13.25 , $P = .01$). In particular, ALT and AST were significantly lower in the second test than in the first test. However, in the control group, there was no statistical difference between the two tests for each liver marker.

As shown in Table 2, only ALT, AST, and GGT were statistically different between the two tests. ROC analyses between the decrease in HBV-DNA and the changes in ALT, AST, GGT are shown in Figure 1 and Table 3. When HBV-DNA load decreased >26 times, ALT was reduced by half or more (sensitivity 62.3%, specificity 78.5%). When HBV-DNA load decreased >63 times, AST was reduced by half or more (sensitivity 62.9%, specificity 79.6%). When HBV-DNA load decreased >76 times, GGT was reduced by half or more (sensitivity 70.7%, specificity 80.4%). In comparison with other liver markers, ALT was more sensitive to a decrease in HBV-DNA load.

4 | DISCUSSION

There are about 400 million people worldwide with HBV infection, and 0.5-1.2 million deaths annually can be attributed to HBV-related liver cirrhosis and cancer.^{7,8} The liver is a target organ of HBV infection. After replication in liver cells, HBV invades into the neighboring cells or damaged host cells and is subsequently released into blood circulation.^{9,10} Measurement of HBV-DNA load can reflect the situation of HBV replication in host cells. Therefore, it can provide a reliable basis for clinical diagnosis and treatments.¹¹ Several studies^{5,6}

TABLE 1 Pearson correlation between HBV-DNA load and liver markers

	<i>r</i>	<i>P</i>
Total protein	.53	.468
Albumin	.133	.031
Albumin/Globulin	.101	.427
Total bilirubin	.025	.136
Direct bilirubin	.037	.591
Indirect bilirubin	.016	.582
ALT	.182	.096
AST	.086	.275
AST/ALT	.008	.983
GGT	.011	.904
Alkaline phosphatase	.007	.893

TABLE 2 Comparison between the twice test results of liver markers in case and control group

Liver function markers	Case group			Control group		
	First test ^a	Second test ^b	P	First test ^a	Second test ^b	P
Total protein (g/L)	71.23±4.63	73.17±4.82	.67	69.37±4.62	72.84±4.59	.27
Albumin (g/L)	42.63±3.83	42.17±4.05	.58	43.51±4.48	44.27±3.87	.36
Total bilirubin (μmol/L)	17.81±8.59	17.24±7.13	.46	18.04±6.23	17.75±8.68	.19
Direct bilirubin (μmol/L)	4.48±1.63	4.01±1.97	.52	4.72±1.83	4.05±1.37	.36
Indirect bilirubin (μmol/L)	14.57±4.28	13.62±5.89	.47	13.85±4.25	15.94±6.83	.28
ALT (U/L)	140.28±38.02	41.63±15.97	.02	120.65±27.28	124.84±29.63	.45
AST (U/L)	127.74±34.63	37.48±11.21	.01	132.71±35.67	130.18±30.17	.41
GGT (U/L)	54.17±20.66	34.95±13.25	.01	54.89±15.14	52.70±17.08	.29
Alkaline phosphatase (U/L)	107.70±25.42	103.96±18.23	.56	105.38±20.35	101.64±17.57	.63

The data which exceeded mean±3SD was ruled out.

^aFirst test: the first results tested in this study of the subjects.

^bSecond test: the second results tested in this study of the subjects.

FIGURE 1 ROC analysis between HBV-DNA increased and the changes of ALT, AST, GGT

performed cross-sectional analysis and reported that liver markers do not always change with changes in HBV-DNA load among CHB patients. However, an association between changes in HBV-DNA load and changes in liver markers is rarely reported.

In this study, we did not find a statistically significant correlation ($P>.05$) between HBV-DNA load and the markers of liver injury (ALT, AST, GGT). Serum albumin and HBV-DNA load were related ($P=.031$); however, there was no significant correlation between them ($r=.13$). Yuan et al.⁵ found that HBV-DNA load and ALT are weakly correlated ($r=.234$, $P<.05$). In addition, Chen et al.⁶ reported that there is no significant relationship between HBV-DNA load and liver markers. Therefore, a lower HBV-DNA load does not necessarily indicate better

liver function. This may be because everyone has a different tolerance and immunity to HBV.

However, for individuals with CHB, a decrease in HBV-DNA load can lead to changes in liver markers. In particular, those whose HBV-DNA load in the second test was >10 times lower than that in the first test showed significant changes in ALT, AST, and GGT. In this study, we found that when HBV-DNA load decreased >26 , >63 , and >76 times, ALT, AST, and GGT were reduced by half or more, respectively. ALT is present in the cytoplasm of liver cells. When HBV copies itself and causes extensive damage to liver cells, ALT will be rapidly released into blood circulation, and it is decreased by a reduction in liver injury caused by a decrease in HBV. This may be because of the

TABLE 3 ROC analysis between HBV-DNA decreased and the changes of ALT, AST, GGT

	Cut-off (times)	Sensitivity	Specificity	AUC	Youden index	P
ALT	26	0.623	0.785	0.734	0.408	.001
AST	63	0.629	0.796	0.742	0.425	.000
GGT	76	0.707	0.804	0.785	0.511	.000

AUC, area under the curve.

higher sensitivity of ALT than other liver markers to a decrease in HBV-DNA load.

In conclusion, among all of the CHB patients, a lower HBV-DNA load does not necessarily indicate better liver function. However, for individuals with CHB, a large change in HBV-DNA load can lead to a significant variation in liver function. In comparison with other liver function markers, ALT was more sensitive to a decrease in HBV-DNA load. It is unfortunate that our study was not able to include participants whose HBV-DNA load changed from low to high because of the insufficient number of participants. Therefore, additional data on participants and clinical studies are required to verify the reliability and validity of our results.

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CONTRIBUTION

Study design: Hua-Bin Wang.

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