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Dynamic monitoring of serum alpha-fetoprotein and its correlation with early hepatocellular carcinoma in patients with chronic hepatitis B.

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Abstract

Objective: To investigate the differences in levels of Alanine Aminotransferase (ALT), HBV-DNA and Alpha-Fetoprotein (AFP) in serum between patients with different clinical types of Chronic Hepatitis B (CHB).

Methods: 274 patients with HBV infection in our hospital from January 2015 to June 2017 were selected and divided into different groups according to various clinical types, including 45 patients in chronic hepatitis B mild group, 62 in chronic hepatitis B moderate group, 58 in chronic hepatitis B severe group, 53 in cirrhosis group and 56 in HCC group. The levels of ALT, HBV-DNA and AFP in serum were detected.

Results: There were significant differences in the levels of ALT, HBV-DNA and AFP in serum between patients in each group (P<0.05). Among them, the serum ALT and HBV-DNA levels in chronic hepatitis B severe group were significantly higher than those in other groups (P<0.05) and the serum AFP level in HCC group was significantly higher than that in other groups (P<0.05). The serum AFP level was positively correlated with the serum ALT and HBV-DNA levels respectively in the patients with chronic hepatitis B, cirrhosis and hepatocellular carcinoma (P<0.05). The analysis of receiver operating characteristic curve showed that when ALT<67.65 U/L, HBV-DNA<3.96 IU/Ml, AFP>96.87 ng/L in distinguishing of severe hepatitis, liver cirrhosis and HCC, HCC in patients could be diagnosed preliminary.

Conclusion: The activity and progression of disease in patients with different clinical types of chronic hepatitis B may be known by the levels of ALT, HBV-DNA and AFP in serum, which is helpful for the early diagnosis of HCC.

Keywords: Chronic hepatitis B (CHB), Hepatocellular carcinoma, Alpha-fetoprotein, Alanine aminotransferase, HBV-DNA.

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Introduction

The incidence of Chronic Hepatitis B (CHB) is rising in the world as one of the chronic diseases which seriously endangers human health, CHB brings suffering to patients and their families and increases social burden of medical treatment [1,2]. Over 240 million people have chronic Hepatitis Virus (HBV) in Africa and Asia, with the highest rates of infection worldwide [3].

Hepatic cirrhosis caused by CHB is accompanied by the pathological processes of persistent necrosis and regeneration of liver cells and destruction of hepatic lobule structure, which make hepatic cirrhosis more likely to develop into hepatocellular carcinoma [4]. The study showed that primary Hepatocellular Carcinoma (HCC) was closely correlated with chronic infection of HBV [5,6]. But the invasion of HCC is relatively latent. Because the early symptoms of HCC are not obvious, when in hospital, many patients may have developed into mid-late stage HCC and lose the opportunity of early treatment. At the same time, poor operative efficacy on advanced hepatocellular carcinoma can lead to a shorter anticipant survival time and a higher case-fatality rate [7].

Therefore, early screening and interference of hepatocellular carcinoma have great clinical significance in reducing mortality rate, extending survival time and improving life quality. Alpha Fetoprotein (AFP) is a glycoprotein synthesized by liver, can be used clinically as an important indicator of severity and progression of liver disease [8]. However, the study suggested that the serum of patients with chronic liver disease and HCC could find the abnormal increase or normality of AFP level and meanwhile, the sensitivity of HCC patients is lower to early HCC diagnosis, so the diagnosis of HCC only according to serum AFP level easily leads to missed diagnosis and misdiagnosis, and has some shortcomings such as lack of sensitivity and specificity and so on [9]. Therefore, how to improve accuracy in the diagnosis of HCC by laboratory testing becomes a hot spot in hepatocellular carcinoma research in recent years. Further study is needed to

investigate dynamic change of serum AFP level in the development process from chronic hepatitis to cirrhosis to HCC, and explore the differences in serum AFP level between patients with different clinical types of CHB and the clinical significance of serum AFP level in early diagnosis of HCC.

Data and Methods

General data

274 patients with HBV infection in our hospital from January 2015 to June 2017 were selected. Inclusive criteria: (1) Diagnostic criteria for CHB and Hepatic Cirrhosis under guidelines for prevention and treatment of chronic hepatitis B (2015) and diagnostic criteria for HCC under guidelines for diagnosis and management of primary liver cancer (2011) should be met [10,11]; (2) All HCC patients were positive for hepatitis B virus surface antigen (HBs-Ag), the final diagnosis time was more than 6 min, and/or the serum HBV-DNA level was more than 1×10^3 IU/L. Exclusive criteria: (1) The patients with hepatic lesion caused by the reasons such as other viral infections, drugs, alcohol and autoimmunity; (2) The patients with concurrent severe diseases in heart, brain, kidney, hematopoietic system and endocrine system and so on; (3) Women during gestation or lactation period. Of 274 patients, there were 162 males and 112 females, aged 38 to 74 y, mean age was 53.27 ± 10.64 y. There were 45 patients in CHB mild group, 62 in CHB moderate group, 58 in CHB severe group, 53 in cirrhosis group and 56 in HCC group. The study had been approved by the Independent Ethics Committee in our hospital, agreed by National Health and Family Planning Commission of China and all the patients were informed and signed the informed consents.

Test method

6 mL of early morning fasting venous blood was collected from subjects, allowed to stand at room temperature for 1 h, and then centrifuged at the rate of 3000 r/min for 10 min. The serum after centrifugation of blood was stored in a refrigerator (-20°C) for later use. (1) The serum AFP level was detected by electrochemiluminescence using Roche E170 electrochemiluminescence immunoassay analyzer and immunoassay kit (Roche Diagnostics, Switzerland), and the test procedures were strictly conformed to the operational rules in the reagent instruction manual. The range of normal reference value is 0~13.6 ng/mL, the upper limit is 1210 ng/mL; (2) The serum HBV-DNA level was detected using SLAN Real Time PCR Detection System 7300 (Real time fluorescence quantitative PCR instrument, America), the reagents (HBV nucleic acid quantitative assay kit) were purchased from German Qiagen bioengineering (Shenzhen) Co., Ltd. and the test procedures were strictly conformed to the operational rules in the reagent instruction manual. The lower limit in sensitivity is 1,000 IU/mL. The main procedures of PCR: Firstly, Double-stranded HBV-DNA is heated to separate it into two single strands during denaturing; then, the temperature is lowered to enable the HBV-DNA primers to attach to the template HBV-DNA in the annealing. Eventually, the new strand of HBV-DNA I is made by the Taq polymerase enzyme while the temperature is raised; (3) The serum ALT level was detected using Hitachi 7180 automatic biochemical analyzer (Japan), the reagents were purchased from Wenzhou Dong'ou Jinma Biotechnology Co., Ltd., and the double-reagent rate method was used. The normal reference value is 0~40 U/L.

Statistical treatment

The statistical analysis was performed using the SPSS 22.0 statistical software. The measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$), the variance analysis was used for comparison among the multiple groups, the SNK-q test was used for comparison between each two groups, the numerical data were expressed as frequency or percentage (%), the chi-square test was used for group comparison, and the correlation analysis was carried out by using Pearson correlation coefficient. Differences with a P value less than 0.05 were considered statistically significant.

Results

Comparison of general data among groups

According to SNK-q or chi-square test, there were no statistical differences in age, gender, body-mass index and other general data among groups (P>0.05) (Table 1).

Comparison of clinical indicator among groups

There were significant differences in the levels of ALT, HBV-DNA and AFP in serum between patients in each group (P<0.05). Among them, the serum ALT and HBV-DNA levels in CHB severe group were significantly higher than those in other groups (P<0.05), and the serum AFP level in HCC group was significantly higher than that in other groups (P<0.05) (Table 2).

Table 1. *Comparison of general data among groups (n).*

Group	Case count	Age	Gender		Body-mass index (kg/m^2)
			Male	Female	
CHB mild group	45	52.81 ± 9.72	26	19	22.93 ± 1.56
CHB moderate Group	62	53.43 ± 10.25	39	23	23.19 ± 1.21
CHB severe Group	58	53.20 ± 10.56	33	25	22.88 ± 1.34
Cirrhosis group	53	54.29 ± 9.27	33	20	23.20 ± 1.44
HCC group	56	53.51 ± 10.19	31	25	23.25 ± 1.39
F or χ^2	-	0.148	1.064		0.839

P	0.964	0.9	0.502

Table 2. *Comparison of clinical indicator among groups ($\bar{x} \pm s$).*

Group	Case count	ALT (U/L)	HBV-DNA (IU/mL)	AFP (ng/mL)
CHB mild group	45	33.21 ± 7.51	2.64 ± 0.72	5.29 ± 1.23
CHB moderate group	62	78.86 ± 18.02[1]	3.43 ± 1.01[1]	45.34 ± 8.62[1]
CHB severe group	58	326.23 ± 82.32[1,2]	5.36 ± 1.63[1,2]	110.16 ± 25.59[1,2]
Cirrhosis group	53	62.67 ± 15.46[1,2,3]	4.70 ± 1.35[1,2,3]	84.82 ± 16.63[1,2,3]
HCC group	56	47.27 ± 12.24[1,2,3,4]	4.29 ± 1.21[1,2,3,4]	209.36 ± 41.15[1,2,3,4]
F		528.932	38.655	569.608
P		0	0	0

Note: [1]When compared with CHB mild group, P<0.05; [2]When compared with CHB moderate group, P<0.05; [3]When compared with CHB severe group, P<0.05; [4]When compared with cirrhosis group, P<0.05.

Correlation analysis of serum AFP level with serum ALT and HBV-DNA levels

The correlation analysis showed that the serum AFP level was positively correlated with the serum ALT and HBV-DNA levels respectively in the patients with chronic hepatitis B, cirrhosis and hepatocellular carcinoma (P<0.05), and the AFP level (HCC) have strongly and positively correlation with the serum ALT and HBV-DNA levels (Table 3).

Table 3. *Correlation analysis of serum AFP level with serum ALT and HBV-DNA levels.*

Item	ALT		HBV-DNA	
	r value	P value	r value	P value
AFP (CHB)	0.32	0.03	0.36	0.01
AFP (Cirrhosis)	0.45	0	0.49	0
AFP (HCC)	0.63	0	0.57	0

Diagnostic efficacy of the serum ALT, HBV-DNA and AFP levels on early HCC

The analysis of results showed that the area under the serum ALT curve was 0.862, the optimum critical value was 67.65 U/L, the diagnostic sensitivity was 81.8% and the specificity was 82.6% (95% confidence interval was 0.754~0.969). The area under the serum HBV-DNA curve was 0.735, the optimum critical value was 3.96 IU/mL, the diagnostic sensitivity was 59.1% and the specificity was 91.3% (95% confidence interval was 0.583~0.887). The area under the serum AFP curve was 0.799, the optimum critical value was 96.87 ng/L, the diagnostic sensitivity was 63.6%, and the specificity was 82.6% (95% confidence interval was 0.672~0.926). When ALT<67.65 U/L, HBV-DNA<3.96 IU/Ml, AFP>96.87 ng/L, HCC could be diagnosed preliminary in distinguishing of severe hepatitis, liver cirrhosis and HCC (Table 4).

Table 4. *Diagnostic efficacy of the serum ALT, HBV-DNA and AFP levels on early HCC.*

Indicator	Optimum critical value	Area under curve	Sensitivity (%)	Specificity (%)	Youden index	95% confidence interval
ALT	67.65 U/L	0.862	81.8	82.6	0.644	0.754~0.969
HBV-DNA	3.96 IU/mL	0.735	59.1	91.3	0.504	0.583~0.887
AFP	96.87 ng/mL	0.799	63.6	82.6	0.462	0.672~0.926

Discussion

Hepatitis B is becoming widespread in China, and its natural infection rate is as high as 42.4 to 80.7 per cent. After infection with HBV, clinical conditions of patients are complicated and diverse, as characterized by various clinical manifestations such as self-limited acute hepatitis, chronic virus carriers, chronic hepatitis, liver cirrhosis, HCC and so on [12]. AFP is a special protein synthesized in the liver during embryonic period. After birth, serum AFP level will decrease rapidly until it disappears. Although extremely low in serum of adults, AFP level, which is clinically often used as serum marker for the diagnosis of HCC, may increase with recovery of liver synthesis capacity after tumorigenesis [13,14].

After observing serum AFP level in patients with chronic HBV at various morbid states, the study showed that the serum AFP

level in CHB mild, moderate, and severe groups gradually increased with progression of disease, the serum AFP level in cirrhosis group was slightly lower than that in CHB severe group, and the serum AFP level in HCC group was significantly higher than that in other groups (P<0.05), which suggested that the serum AFP level has a certain reference value in the early diagnosis of HCC [15]. In addition, the results indicated that the serum AFP level was also increased after liver lesion. It is generally believed that the increase of serum AFP level in CHB patients is caused by the regeneration of liver cells after necrosis, and the continuous and abnormal increase of serum AFP level in CHB patients often indicates a high risk of carcinogenesis. The study of Chunhua et al. suggested that although the serum AFP level in patients with CHB and cirrhosis may be increased, its abnormal increase is more common and obvious in HCC patients [16]. On the other hand, some clinical features of patients could lead to false-negative message on serum AFP level, such as the near-normal differentiation of cancer cells in the early stage of HCC, the encasement of degenerative necrotic tissue by connective tissue, excessive cancer tissue in connective tissue and so on [17]. Therefore, the diagnosis of HCC only according to serum AFP level easily leads to missed diagnosis and misdiagnosis [18,19].

ALT mainly present in hepatic cytoplasm is an enzyme involved in protein metabolism in humans. When tissues or organs of the body are active or diseased, especially when suffering from hepatitis or cirrhosis, ALT is released into blood, which leads to a increase of serum ALT level. Therefore, ALT is an important indicator of the extent of liver disease [20,21]. The results showed that the serum ALT level was significantly different among groups and the serum ALT level in the CHB severe group was significantly higher than that in the other groups (P<0.05), which was consistent with the results of Xiangming et al. Occurrence of HCC was closely correlated to persistent infection of HBV [22]. Mendy et al. suggested that continuous internal replication of HBV-DNA is a high risk factor for HCC and deletion and point mutation of tumor suppressor gene *p53* allele may lead to carcinogenesis [23]. The results showed that the serum HBV-DNA level was significantly different among groups, and the serum HBV-DNA level in the CHB severe group was significantly higher than that in the other groups (P<0.05). In the course from CHB to liver cirrhosis to HCC, the activity of HBV replication declines gradually. Immune clearance during this process can cause necrosis of liver cells leading to inhibition of virus replication which is dependent on liver cells [24,25].

At present, two or more tumor markers are commonly tested jointly in clinical trials to improve the accuracy of diagnosis of HCC. Xiping et al. tested jointly Golgi protein 73 (GP73) and AFP in the diagnosis of PHC, and the results showed that the sensitivity in the joint testing was 79.6%, the specificity was 80.3%. The sensitivity in the joint testing is higher than that in the individual testing of GP73 (72.7%) or AFP (49.8%), the specificity in the joint testing was higher than that in the individual testing of GP73 (70.8%) and lower than that in the individual testing of AFP (95.9%). In this study, 67.65 U/L, 3.96 IU/mL and 96.87 ng/L were set respectively as the optimum critical values of ALT, HBV-DNA and AFP, the sensitivities of the three indicators were 81.8%, 59.1% and 63.6% respectively, and the specificities were 82.6%, 91.3% and 82.6% respectively. The above-mentioned results indicated that the accuracies of diagnosis of HCC were different using ALT, HBV-DNA or AFP in serum respectively. Thus, when diagnosing HCC, this study also explored the effectiveness of ALT, HBV-DNA and AFP. The results showed that in the process of diagnosing HCC preliminary, when ALT<67.65 U/L, HBV-DNA<3.96IU/Ml, AFP>96.87ng/L in distinguishing of severe hepatitis, liver cirrhosis and HCC, HCC can be highly suspected. While the results of co-related analysis in this study showed, serum AFP level in patients with CHB, liver cirrhosis and HCC was positively in correlation with ALT and HBV-DNA level (P<0.05). The above-mentioned results indicated that the serum ALT and HBV-DNA levels were closely correlated with the serum AFP level in patients with different clinical types of CHB. Ji et al. concluded that the more solid evidence for HCC surveillance, treatment and monitoring would be offered by AFP and DCP level, and which is similar with this study [26,27].

Conclusion

There is difference in levels of ALT, HBV-DNA and AFP in serum between patients with different clinical types of CHB, and the difference is increased gradually with aggravation of conditions of patient. In the identification of CHB, liver cirrhosis and HCC, the activity and progression of liver disease need to be judged and determined according to expression levels of different indicators and conditions of patient. The ALT, HBV-DNA and AFP in serum are beneficial to the improvement of early HCC diagnosis.

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