Quantitation Detection of Hepatitis B Surface Antigen and Its Significance in Patients with Hepatic Cirrhosis

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ABSTRACT Objective: To assess the clinical significance of the quantitation of HBsAg in HBV-associated hepatic cirrhosis. Method: Sixty HBV-associated hepatic cirrhosis patients were included in the study. The patients were divided into the compensated cirrhosis group (n = 35) and the decompensated cirrhosis group (n = 25) based on the diagnostic criteria of hepatitis determined at the 10th National Conference on Viral Hepatitis (Xi'an, September 2000). 20 asymptomatic healthy hepatitis B carriers were recruited as the control group. The serum titers of HBsAg and HBeAg were determined using electrochemiluminescence immunoassay (ECLIA), and HBV DNA load was measured using immunofluorescence quantitative polymerase chain reaction assay(PCR). Result: HBsAg level in the control group, compensated cirrhosise group and decompensated cirrhosis group were 2574.73 ± 3252.27 COI, 5494.35 ± 2129.84 COI and 6921.25 ± 1957.60 COI, respectively. The differences among the three groups were statistically significant (P<0.05). In compensated cirrhosis group, the pearson correlation in HBsAg vs. HBV DNA and HBsAg vs. HBeAg were significantly reverse correlation (P<0.05), with r values of -0.350 and -0.514 respectively. In decompensated cirrhosis group, HBsAg had no significant correlation with HBV DNA or HBeAg, with r values of -0.020 and 0.154 respectively. Conclusion: HBsAg level is significantly higher in decompensated cirrhosis group than in compensated cirrhosis group, it is also higher in compensated cirrhosise group than in control group. With the progression of liver disease, a rising HBsAg gradient is observed. In compensated cirrhosis group, HBsAg level has a significantly reverse correlation with HBV DNA and HBeAg, hence HBsAg is a indirect index to reflect viral replication. In decompensated cirrhosis, HBsAg has no significant correlation with HBV DNA and HBeAg, suggesting that HBsAg does not reflect the activity of viral replication.

Key words: HBsAg; HBV DNA; HBeAg; Hepatic cirrhosis

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Hepatitis B surface antigen (HBsAg) is the outer membrane component of virus particles, encoded by the S gene of outer membrane proteins [1]. HBsAg is the earliest serological marker in patients infected with HBV virus, whereas HBsAg serological conversion is the gold standard of hepatitis B patients cure. Hepatitis B virus covalently closed circular DNA (HBV CCCDNA) is the original template of HBV preRNA transcript, and a key factor for the persistent infection with hepatitis B virus. It is therefore considered the best specific replication index of hepatitis B virus. However, monitoring HBV CCCDNA level in patient liver tissue through biopsy is a difficult procedure. Therefore it is important to find a non-invasive alternative that can reflect the level of HBV CCCDNA. It has been reported that HBVcccDNA is correlated with serological HBsAg level and HBV DNA load [2-5], and that HBsAg level can be used as an alternative indicator. The object of this study is to assess the correlation of HBsAg level with HBV DNA load and HBeAg level in patients with hepatic cirrhosis, in order to find an inexpensive and convenient surrogate marker of viral replication of HBV as an important supplement for the detection of HBV DNA.

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1 Material and methods

1.1 Patients

We recruited 60 patients with HBV-associated hepatic cirrhosis and 20 asymptomatic healthy carriers of hepatovirus B who were diagnosed at the Department of Infectious Disease, Gastroenterology and Hepatology, the affiliated Hospital of Qingdao University medical college. All patients had been positive for HBsAg for at least 6 months. The patients were diagnosed based on the diagnostic criteria of hepatitis determined at the 10th National Conference on Viral Hepatitis (Xi'an, September 2000). The cases were divided into three different groups according to the diagnostic criteria, the compensated cirrhosis group (n = 35), the decompensated cirrhosis group (n = 25) and the control group (n = 20). Each patient agreed to join in the experiment and signed the informed consent. Patients with the following complications were excluded: (1) autoimmune hepatitis, (2) Wilson's disease, (3) primary biliary cirrhosis, (4) co-infections such as HAV, HCV, HDV and HEV, (5) toxic hepatitis, (6) alcoholism, (7) a past history use of antiviral agents, (8) used of liver protectant in the last six month, (9) other diseases such as immune disease, diabetes, hyper-

tension, lung disease as well as other locum tumor.

1.2 Detection of parameters

Serum samples were collected simultaneously at the time of admission. Serological markers of HBsAg and HBeAg were determined using the method of electrochemiluminescent immunoassay (ECLIA) and the results were analyzed by Roche automated analysers Elec-sys2010. HBV-DNA load was measured with the immunofluorescence qualitative polymerase chain reaction assay (PCR).

1.3 Statistical analysis

Statistical analysis was carried out with Statistical Package for Social Science program version 11.5 (spss 11.5) for Windows. The quantitative data were presented in the form of mean and standard deviation (SD). One-way ANOVA tests was used to compare parameters among the three groups, and P values less than 0.05 was considered statistically significant. Correlations were analyzed by Pearson's correlation coefficient (r), and P values less than 0.05 were deemed statistically significant. The quantitative values of HBV DNA and HBeAg were used the logarithmic values, because the initial data of them were nonnormal distribution data.

2 Results

2.1 Quantitation of HBsAg、HBV DNA and HBeAg

The serum titer of HBsAg was the lowest in asymptomatic HBV carriers, higher in the compensated cirrhosis group, and the highest among the decompensated cirrhosis group. The differences among the three groups were statistically significant (P<0.05, table 1).

HBV DNA load was the highest in asymptomatic HBV carriers，lower in the compensated cirrhosis group, and the lowest among the decompensated cirrhosis group. However, the differences of HBV DNA among the three groups were not statistically significant (Table 1).

Similarly the HBeAg titer was the highest in asymptomatic HBV carriers, lower in the compensated cirrhosis group, and the lowest among the decompensated cirrhosis group. And only the difference between asymptomatic HBV carriers and the decompensated cirrhosis group was statistically significant (P<0.05, Table 1).

Table 1 HBsAg, HBV DNA and HBeAg quantitation(mean± SD)

Group	cases	HBsAg(COI)	HBV DNA(copies.ml-1)	HBeAg(COI)
Asymptomatic HBV carrier	20	2574.73± 3252.27*	5.69± 2.38	1.63± 1.86
Compensated cirrhosis	35	5494.35± 2129.84*	5.34± 1.24	0.88± 1.49
Decompensated cirrhosis	25	6921.25± 1957.60*	5.29± 0.90	0.22± 0.93△

Note :* P<0.05 Comparison among three groups, △ P<0.05 compared with carriers

2.2 Correlation of HBsAg level with HBV DNA load and HBeAg level

Among the compensated cirrhosis group, the level of HBsAg was reverse correlated with HBV DNA and HBeAg; Among the decompensated cirrhosis group, the level of HBsAg was not significantly correlated with HBV DNA or HBeAg.

Table 2 Correlation of HBsAg with HBV DNA and HBeAg

Group	Case	HBsAg and HBVDNA		HBsAg and HBeAg	
		r	p	r	p
Compensated group	35	-0.350	0.048	-0.514	0.002
Decompensated group	25	-0.020	0.927	0.154	0.473

3 Discussion

HBsAg is the outer membrane protein consisting of a mixture of glycoproteins. There are three types of virus particles in the blood of patients with HBV infection, dane particles, spheroidal particle, and tubiform particle. Dane particles are intact virus particles, consisting by HBV DNA and HBsAg. Spheroidal particles and tubiform particles are outer membrane particles containing only by HBsAg. Spheroidal particles are the highest in serum, whereas dane particles are the lowest. Even in patients with active viral replication, dane particles containing the core of virus (viral particles) is well below the level of outer membrane particles (spheroidal particles and tubiform particles). Therefore, HBsAg is often measured as a sign of HBV infection.

Previous detection of HBsAg was limited to the qualitative determination. With the development of detection technology, quantitation have been gaining more attention clinically. Quantitative detection of HBsAg is simple, rapid, accurate, sensitive, specific and reproducible. More importantly, recently researchers suggested that serum HBsAg and intrahepatic HBV cccDNA levels were significantly associated [4]. And there is studies showing that HBsAg levels in serum and intrahepatic HBV cccDNA decline si-

multaneously in response to anti-viral therapy [2,7]. In recent years, monitoring the level of serum HBsAg during anti-viral therapy has become the focus of attention. During the course of treatment using nucleoside analogues, the decrease in the level of HBsAg coincide with the decrease of HBV DNA [3, 8-10]. But the increase of serum HBsAg usually occurs before HBV DNA rebound often and biochemics breakthrough (ALT levels Increased) when resistant strains appear [3,11,12]. HBsAg is also monitored in response to interferon treatment. In the cases where HBsAg level decreases rapidly and dramatically early during the treatment, the prognosis favor sustained virulogy responder (SVR) and HBeAg seroconversion in response to interferon [6,13,14].

This study shows that HBsAg level in decompensated cirrhosis group is significantly higher than compensation group (P<0.05), which is then higher than the carrier group (P<0.05). It suggests that with the progress of liver disease, HBsAg gradient rises with the mean level higher in more severe liver disease. A possible explanation is that HBV CCCDNA is the expression template of HBsAg in liver cell. With the history of extended existence of HBV virus, the number of liver cells infected rises, which then lead to increased number of HBV CCCDNA in liver cells [15]. Another factor is that higher HBsAg easily induce to immunizing active phase and lead to more severe liver damage[16]. There has been similar reports about the relationship between HBsAg and liver disease, where the intracellular localization of HBsAg was associated with the grade of liver disease [17]. The authors concluded that patients with serious inflammation and histological activity had higher hepatic cellular membrane HBsAg.

HBV DNA and HBeAg are recognized as an important indicator of virus replication. In this study, HBV DNA and HBeAg are the highest in control group, the second in compensated cirrhosis group, the lowest in decompensated cirrhosis group. That means the virus is hyper-replicating in control group, and hypo-replicating in decompensated cirrhosis group.

In liver cirrhosis compensated group, the level of HBsAg is reverse correlated with HBV DNA and HBeAg. The result is consistent with previous reports in chronicity HBV patients [1,18]. The possible reasons for the reverse correlation mechanism are as follows. First, when the virus strain generating HBV CCCDNA in liver become the dominant strain, the other virus strains become relatively inactive [19], that lead to a reduction of the total HBV DNA. But the generation of HBV CCCDNA is relatively higher and the formation of HBsAg also increases. Second, the synthesis of HBV CCCDNA is regulated by negative feedback of HBV virus, when the virus is in low copy state, the mature virus is primarily used to supplement the HBV CCCDNA pool [20]. Third, the PCR detection of HBV DNA is considered as the diagnositic "gold standard", but PCR is used to detect the replicating virus only[21]. HBV CCCDNA is a template to produce HBsAg, and HBsAg is not restricted by viral replication state. These results implied that, in liver cirrhosis compensated group, serum HBsAg level and HBV load is not associated. When viral replication is inactive, HBsAg level is high. HBsAg level is all affected by the viral replication state, so HBsAg level indirectly reflect viral replication. In the decompensated cirrhosis group, there is no correlation in HBsAg and HBV DNA, or HBsAg and HBeAg. Therefore, in decompensated cirrhosis, HBsAg does not reflect the level of viral replication.

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肝硬化患者乙肝表面抗原的定量检测及意义

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摘要 **目的**：探讨 HBsAg 定量测定在乙肝相关性肝硬化病程中的变化和意义。**方法**：选择乙肝相关性肝硬化患者 60 例纳入实验对象，根据 2000 年 9 月(西安)第 10 次全国病毒性肝炎学术会议修订的《病毒性肝炎防治方案》中的诊断标准分为代偿期组和失代偿期组，其中代偿期组 35 例，失代偿期组 25 例。另选取 20 例乙型肝炎病毒携带者作为对照组。应用电化学发光免疫分析法测定患者血清中 HBsAg 和 HBeAg 滴度，免疫荧光定量 PCR 法检测 HBV DNA 载量。**结果**：对照组、肝硬化代偿期组和肝硬化失代偿期组 HBsAg 滴度分别为：2574.73± 3252.27 COI、5494.35± 2129.84 COI 和 6921.25± 1957.60 COI，三组之间差别均有统计学意义 (P<0.05)。肝硬化代偿期组中，HBsAg 滴度与 HBV DNA、HBeAg 水平呈负相关性 (P<0.05) (r=-0.350；r=-0.514)。肝硬化失代偿期组中，HBsAg 滴度与 HBV DNA 及 HBeAg 水平均无明显相关性 (r=-0.020；r=0.154)。**结论**：肝硬化失代偿期 HBsAg 滴度明显高于肝硬化代偿期，代偿期 HBsAg 滴度高于 HBV 携带者组，即 HBsAg 滴度随肝脏疾病进展呈阶梯型递增。肝硬化代偿期，HBsAg 滴度与 HBV DNA、HBeAg 水平呈负相关性，HBsAg 水平可以作为评估病毒复制的参考指标。肝硬化失代偿期，HBsAg 滴度与 HBV DNA 和 HBeAg 无相关性，不能反映病毒复制水平，不能作为评估病毒复制的参考指标。

关键词：HBsAg；HBV DNA；HBeAg；肝硬化

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