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Evaluation of the COBAS® TaqMan® system in patients with low hepatitis B virus DNA undetectable with PCR assay kit

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Abstract. – OBJECTIVE: We conducted a comparison of the diagnostic kit for quantification of hepatitis B virus DNA (PCR-fluorescence probing) and COBAS TaqMan automated nucleic acid extraction and real-time polymerase chain reaction (PCR) amplification systems. We tested their capacity to quantify and diagnose patients with chronic viral hepatitis B with low viral load < 1 x 10^3 IU/mL, in hope to provide further evidence for promoting the application of COBAS TaqMan as the diagnostic method for such patients.

PATIENTS AND METHODS: Diagnostic kit and COBAS TaqMan system were tested on 100 patients diagnosed with chronic viral hepatitis B in our hospital and with a viral load lower than the detection limit of real-time extraction-quantification kit. These patients included 47 cases with chronic viral HBV, 53 cases of HBV-associated cirrhosis (11 cases were HBV-associated liver cancer with cirrhosis). COBAS TaqMan real-time quantification PCR with a sensitivity of 20 IU/ml was performed to test the reproducibility for the diagnosis result.

RESULTS: The COBAS TaqMan real-time system quantified 76 cases out of 100 with a viral load higher than 20 IU/ml (detection rate, 76%). Among these patients, there were 33 cases of chronic viral HBV (without cirrhosis) (detection rate, 70.2%), 43 cases of cirrhosis (detection rate, 81.1%, including 28 cases of compensatory cirrhosis and 15 cases of decompensated cirrhosis), and 11 cases of liver cancer (detection rate, 81.2%).

CONCLUSIONS: The COBAS TaqMan system has higher sensitivity than traditional real-time PCR detection kit, especially for HBV-related cirrhosis and liver cancer with low viral load. The limitation of real-time PCR should be taken into account during treatment monitoring and the alternative of COBAS TaqMan system should be promoted in patients with high risk of liver cirrhosis and cancer to avoid delayed diagnosis and improve clinical outcome.

Key Words:
COBAS TaqMan system, HBV with low virus load, Hepatitis B, Liver cirrhosis, Liver cancer.

Introduction

Hepatitis B virus (HBV) infection is a global public health problem, while in China HBV infection is highly endemic. The seroepidemiological survey on HBV infection conducted in 2006 showed that HBsAg carrier rate was 7.18% in the overall Chinese population, and 0.96% in children under 5 years old[1,2]. Accordingly, there were an estimated 93 million HBV carriers, among which around 20 million were infected with chronic hepatitis B. According to the data of World Health Organization (WHO), hepatitis B causes about 1 million deaths of HBV-related liver failure, cirrhosis, and hepatocellular carcinoma annually[3,4]. 6 to 20% of chronic hepatitis B can progress to cirrhosis within 5 years[5], and persistent viral replication is a major risk factor that contributes to progression to cirrhosis and hepatocellular carcinoma.

Hepatitis B virus DNA level is the most direct indicator reflecting the HBV replication activity and infectivity; it is also one of the important indexes to observe the efficacy of antiviral therapy, prognosis and guide the application of antiviral drugs. Elevated serum HBV DNA level is a strong risk predictor of hepatocellular carcinoma independent of HBeAg, serum alanine aminotransferase level, and liver cirrhosis[6]. Quantitative detection of DNA HBV has broken the limitation of the indirect methods, such as immunological method, by directly reflecting the virus load of patients with virus nucleic acid. It has been pointed out by the latest version of guidance released by liver disease association in China, Asia-Pacific, Europe and America that inhibition of hepatitis B virus replication proactively and effectively is the key to delaying the progression of the disease and improving clinical outcome. Early initiation of active antiviral therapy

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serves the benefit of patients, especially those with liver cirrhosis and liver cancer[7]. Therefore, early and accurate assessment of viral replication status is the cornerstone for early treatment.

COBAS TaqMan automated nucleic acid extraction and real-time polymerase chain reaction (PCR) amplification system have been developed recently for HBV DNA detection and quantification. Before being widely adopted, these new extraction-quantification systems must be tested for their sensitivity and reproducibility on sample panels representing chronic viral hepatitis B patients with low viral load. The COBAS system, compared with the traditional HBV PCR reagent, better reflects the viral replication with higher sensitivity and lower quantitative limit, which is of great significance for the diagnosis, treatment and prognosis of HBV-related diseases. The performance for detecting HBV DNA by Cobas Taqman assay has been published[8,9]. Moreover, to the best of our knowledge, the system has not been tested among Chinese patients with low HBV DNA load to compare its performance with traditional PCR assay kit currently conducted in hospitals. In this article, we selected patients diagnosed in our hospital with a low viral load to test these patients with the traditional PCR and COBAS system to compare the viral replication under different disease states.

Patients and Methods

Patients

Patients with chronic hepatitis B diagnosed in the First People's Hospital of Yongkang City from January 2013 to June 2015 were selected. The patients should meet the following inclusion criteria: (1) Hepatitis B surface antigen test positive and the HBV DNA load $< 1 \times 10^3$ IU/ml; (2) Patients are native to anti-hepatitis B treatment, or patients are diagnosed with chronic viral hepatitis B with repeated tests confirmed abnormal liver function, HBV infection-related cirrhosis, or chronic HBV-infection complicated with liver cancer; (4) Patients with other liver conditions, such as Hepatitis C, autoimmune liver disease, Hepatitis A, Hepatitis E, are excluded from subjects group. A total of 100 cases were recruited, including 69 male and 31 female cases. Among the subjects, 47 are diagnosed with chronic hepatitis B, 33 with liver cirrhosis, and 11 with liver cancer. The age of subjects ranged from 20 to 68. For subjects with HBV DNA test results with traditional QF-PCR below 1×10^3 IU/ml, their serum samples were tested again with COBAS Taqman system Table I shows the general characteristics of the tested subjects.

Methods

Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR) System

The reactions were carried out using real-time PCR equipment (StepOne Real-Time PCR System, Applied Biosystems). QF-PCR assay kit for detection of HBV DNA was purchased from Shanghai Fuxing High Tech Co. And the extraction, amplification, and detection of HBV DNA in serum samples were conducted according to the manufacturers' instruments.

COBAS® TaqMan® System

TaqMan technology uses double-marked fluorescent hybridization probes that bind specifically to the sample between primers. The COBAS TaqMan test quantifies the amplicons during the exponential phase of amplification. The appearance of the specific fluorescent signal is consid-

Table I. General characteristics of the tested subjects.

Parameters		N
Age (yrs)	With liver cirrhosis:	51.63 ± 10.2
	With chronic hepatitis B	41.12 ± 11.99
Gender	With liver cirrhosis:	Male, 32; Female, 11
	With HBV:	Male, 20; Female, 13
Chronic hepatitis B	47	
Liver cirrhosis	53	
Liver cancer	11	
HBeAg+	8	
HBeAb-	66	

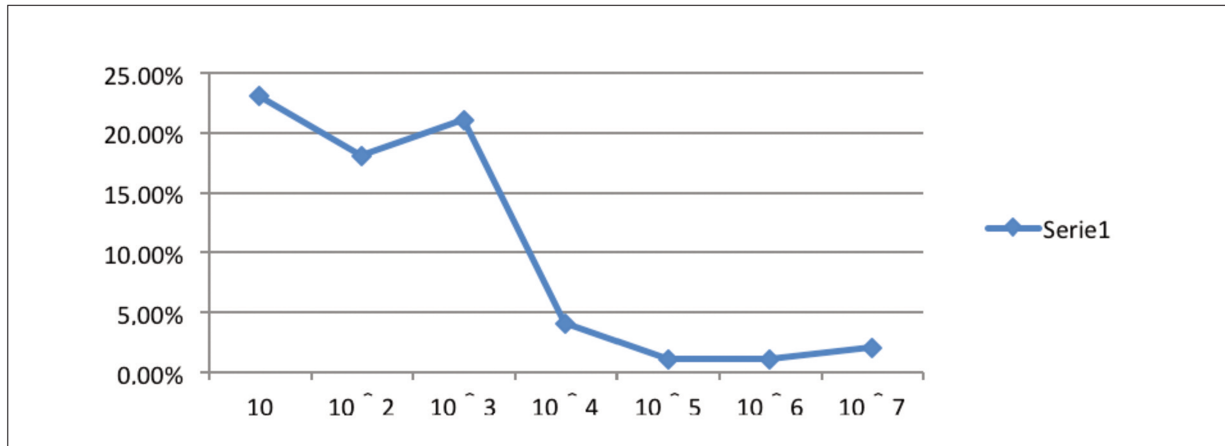

Figure 1. Distribution of detected HBV DNA load.

ered a critical threshold value (Ct). The Ct is defined as the number of fractional cycles in which the fluorescence emitted by the sample exceeds a preset threshold (assigned level of fluorescence) and marks the beginning of an exponential growth phase of this signal[10]. The tests were conducted independently by ADICON Clinical Laboratories, Inc with COBAS TaqMan system strictly according to the manufactures' instruments.

Statistical Analysis

The data was analyzed with SPSS 15.0 (SPSS 15.0 for Windows, SPSS Inc., Chicago, IL, USA). The quantitative data was expressed as mean and standard deviation (± SD). Comparison between two groups was tested with independent *t*-test, and Wilcoxon test was used for logarithmic comparison. Count data was expressed in percentage (%) and tested with the chi-square test. Statistical significance was set at $p < 0.05$.

Results

76 out of 100 subjects tested with COBAS TaqMan system were confirmed with a low load of HBV virus ranged from 2.42E+01 IU/ml to 1.13E+06 IU/ml. The total detection rate was 76%, and the distribution of HBV DNA was showed as Figure 1.

The detection rates for chronic hepatitis B patients, liver cirrhosis and liver cancer patients are 70.2% (detected 33 out of 47 cases), 81.1% (detected 43 out of 53 cases), and 81.2% (detected 9 out of 11 cases) (Table II). No statistically significant difference was detected between detection rates of chronic hepatitis B and liver cirrhosis ($p = 0.202$), or between the detection rate of cancer and non-cancer patients ($p = 0.436$). The detection rates for chronic hepatitis B, cirrhosis, and liver cancer are comparable.

24% of the tested patients are with a viral load lower than the detection limit of both traditional PCR assay kit and COBAS TaqMan system. Arranged by the gender, the detection rates of viral load for male and female patients are 75.4%

Table II.

	Detected	Not detected	χ^2	*p*
Males	52	17	0.05	> 0.05
Females	24	7		
Chronic viral hepatitis B	33	14	1.628	0.202
Liver cirrhosis	43	10		
With liver cancer	11	2	0.608	0.436
Without liver cancer	65	22		
HBeAg +	7	1	0.13	> 0.05
HBeAg-	69	23		

(detectable in 52 out of 69 male subjects) and 77.4% (detectable in 24 out of 31 female subjects) (Table II). The detection rates for males and females are comparable ($p = 0.824$).

Among the 92 subjects with negative HBeAg, 69 can be detected with COBAS TaqMan (detection rate, 75%), and the detection rate for patients with positive HBeAg is 87.5% (7 detected out of 8) (Table II). The detection rates for patients with positive and negative HBeAg are comparable (0.217).

Discussion

According to the test results, the COBAS® TaqMan® HBV Test satisfies the requirements for reliable quantification of HBV DNA in clinical specimens and the detection rates for all sample panels are comparable, reflecting desired detection stability for samples with low HBV viral load compared with traditional PCR assay kit.

DNA HBV load is the key index for clinical evaluation and monitoring of HBV replication, and is used clinically for diagnosis of chronic hepatitis B virus infection, selection of indications for antiviral therapy and evaluation for therapeutic effect. Clinical evidence has shown that the incidence and mortality of chronic hepatitis B are related to persistent viral replication, which also contributes to its development into cirrhosis and/or liver cancer[11,12]. The HBV DNA load closely relates with clinical outcome of chronic hepatitis B independent of the state of HBeAg, virus genotype, baseline AL level, and other risk factors. The low viral load of HBV DNA is also a risk factor for the development of HCC[13]. Therefore, it is of practical value for us to improve test methods for quantitation of HBV DNA replication.

Currently, a variety of gene detection techniques, both domestic and imported commercial kits, with various tests methods, reagents, sensitivity and detection range are available. It is acknowledged that the sensitivity and performance of domestic real-time PCR assay kits for HBV DNA detection was outperformed by imported equivalents. Viral load testing remains the gold standard for the management of HBV antiviral therapy, and for now, the COBAS AmpliPrep-COBAS TaqMan real-time polymerase chain reaction assay has been regarded as the gold standard method for detecting HBV DNA by providing accurate and reproducible results at the key medical decision points, allowing the clinician to optimize patient outcomes. Compared with traditional FQ-PCR assay kits widely used by Chinese hospitals, the superiority of COBAS Amplicor HBV DNA quantitative detection mainly reflected among these with low viral load, which may end up with false negative results with traditional methods. As confirmed in this study, the selected subjects with low HBV DNA have been tested again with COBAS TaqMan system. The detection rate was improved to 76% with the detected HBV DNA mainly distributed between 100 to 1000 IU/ml. Therefore, we concluded that the target system applies to the population of the repeated abnormal liver function and the patients of liver cirrhosis or liver cancer. Besides, the detection rates are high among all sample panels without any statistical significant difference, especially for patients with cirrhosis/liver cancer, whose detection rate was up to 80%. The test results showed no significant gender difference and independent of the HBeAg state. Moreover, the detection rate for patients with positive HBeAg was high up to 87.5%, suggesting that we should promote its application for such population.

Conclusions

To sum up, we validated the COBAS TaqMan system with desired results in patients with chronic hepatitis B, liver cirrhosis or liver cancer complicated with repeated abnormal hepatic function results. Therefore, COBAS TaqMan system should be further promoted among the tested population for early knowledge of viral load, early diagnosis and treatment. This is the first experiment to study the performance of COBAS TaqMan system to test HBV DNA among patients with low viral load despite of its limited sample volume. Still expanded trial involving cases is expected to carry out to further validate the conclusions.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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