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Comparison of Quantitative Real-Time PCR and CLIA for the Detection of Hepatitis B Virus (HBV)

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Abstract: To investigate the value of quantitative real-time polymerase chain reaction (Real-Time PCR) and chemiluminescence method (CLIA) for the detection of hepatitis B virus (HBV). 321 HBV-DNA-positive patients from November to December 2023 were selected for the study objects, with the fasting blood collected from the patients for centrifugal serum separation. Then, Real-Time PCR and CLIA tests were performed to count the positive results of different methods and the copy number of Real-Time PCR. Among the 321 patients, there were 220 positive cases of HBsAg+, HBeAb+ and HBcAb+, the largest number that accounted for 68.32%. The mean values of different HBV-DNA loads varied, with the highest values for medium and high loads of HBsAg+, HBeAg+ and HBcAb+, and the highest values for low loads of HBsAb+, HBsAg+, HBeAb+ and HBcAb+. CLIA and Real-Time PCR correlate well with HBV laboratory tests, which can assist in the early diagnosis of hepatitis B and provide a favorable reference for the early treatment of the disease.

1. Introduction

Hepatitis B virus (HBV), a globally widespread virus that is mainly transmitted through blood and body fluids, is one of the major pathogens causing serious liver disease [1]. HBV infection is a major public health problem worldwide that causes millions of infections and hundreds of thousands of deaths each year [2]. Currently, testing methods for hepatitis B virus include traditional serological and molecular biological detection methods, with serological detection methods mainly relying on the detection of specific antibodies and antigens in the patient's serum [3]. Although these methods have a high degree of accuracy and reliability, they do not provide continuous monitoring due to the limited ability to accurately localize the course of infection and the influence of fluctuating antibody/antigen levels in the blood [4]. The Quantitative Real-Time polymerase chain reaction method (Real-time PCR) is a highly sensitive and specific molecular biology assay that can directly detect viral nucleic acids, with an advantage in the early detection of infection. The Chemiluminescence method (CLIA) is a highly sensitive and rapid method that uses a labeled antibody to bind to a specific antigen and emit a chemiluminescent signal [5]. The comparison of

these two methods provides more options and guidance for accurate monitoring of hepatitis B virus. In the study, the value of Real-Time PCR and CLIA as two methods for detecting hepatitis B virus was compared to evaluate the relevance of the different assays in monitoring HBV infection, so as to provide certain references for the diagnosis and treatment of diseases, as reported below.

2. Information and Methods

2.1 General information

A total of 321 HBV-DNA-positive patients admitted from November 2023 to December 2023 were selected for the study. Inclusion criteria: patients aged 18 years and above; patients with complete medical records; patients who agreed to participate in the study and signed the informed consent form. Exclusion criteria: patients with other chronic liver diseases (e.g. hepatitis C); patients with immune system abnormalities, autoimmune diseases or other serious chronic diseases; patients who have had liver surgery or transplantation in the past 6 months; patients who refused to participate in the study or who were unable to collect blood samples in accordance with the requirements of the study; patients with poor compliance and patients who withdrew from the study. The baseline data of the study subjects were as follows: 199 males and 122 females aged 19-85 years, with a mean of (43.37 ± 11.91) years. The study was approved by the Medical Ethics Committee of the hospital.

2.2 Methods

2.2.1 Sample collection and processing

All patients were collected in accordance with the requirements of blood specimens, with the patients kept in a fasting state before blood collection, which was carried out in the morning. 2-3 ml of venous blood were collected and placed in a blood collection tube containing separator gel and procoagulant. The blood specimen was sent for immediate processing. After complete coagulation of the blood sample, the serum was separated by centrifugation at 3000 r/min for 10 min and tested immediately. The serum of the samples that cannot be detected in time should be stored in the refrigerator at -20℃ for examination.

2.2.2 Detection methods

RT-PCR detection: High-throughput real-time fluorescence quantitative PCR (ABI 7500, Thermo Fisher Scientific, USA) was used to detect the HBV-DNA level in serum, with the relevant reagents purchased from Guangzhou Da'an Gene Technology Co., Ltd. The test was carried out strictly according to the instructions of the kit, with blank, negative and positive controls set in each experiment. Negative control, positive control and sample DNA were added into the relevant reaction tubes and placed in the PCR amplifier for amplification. The amplification conditions were pre-denaturation at 93℃ for 2min, then pre-denaturation at 93℃ for 45s and 55℃ for 60s of 10 cycles, after which pre-denaturation was performed at 93℃ for 30s and 55℃ for 45s of 30 cycles. At the end of the reaction, the quantitative DBV-DNA results were obtained automatically by computer.

CLEIA detection: Five indicators of hepatitis B in serum, including hepatitis B surface antibody (HBsAb), hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HBcAb), hepatitis B e antibody (HBeAb), and hepatitis B e antigen (HBeAg) were detected, with the corresponding indexes detected by automatic immune analyzer (ARCHITECT i2000sr, Abbott) and the supporting reagents referring to the instructions of the reagent kits.

Indoor quality control was carried out daily, and the relevant operations were performed in strict accordance with the instructions of the instruments and reagents, with each monitoring batch containing the corresponding negative and positive reference products.

2.3 Observation indicators

(1) Positive detection. The positive specimens for HBV-DNA were counted for detection based on the five kinds of hepatitis B antigen-antibody, and they were grouped into different positive conditions to obtain detailed distribution of positive results.

(2) Comparison of test results. The positive results of CLIA for different groups were compared and the Real-Time PCR copies were counted.

2.4 Statistical methods

SPSS21.0 software was used to analyze the statistical results, with the count data expressed as (%). In addition, the $\chi 2$ test was applied in the study, with $P<0.05$ indicating a statistically significant difference.

3. Results

3.1 Hepatitis B 5-positive detection results

Hepatitis B positive was detected, among which HBsAg+, HBeAb+, and HBcAb+ had the highest number of positive cases (220 cases), accounting for 68.32%, followed by HBsAg+, HBeAg+, HBcAb+ (79 cases), accounting for 24.53%. See Table 1 for details.

Table 1: Statistics of HBV-DNA positive detection results and Hepatitis B 5 positive tests

Serologic marker	Cases	Percentage (%)
HBsAg+/HBeAg+/HBcAb+	79	24.53%
HBsAg+/HBeAb+/HBcAb+	220	68.32%
HBsAg+/HBeAg+/HBeAb+/HBcAb+	7	2.17%
HBsAg+/HBsAb+/HBeAg+/HBcAb+	7	2.17%
HBsAg+/HBsAb+/HBeAb+/HBcAb+	5	1.55%
HBsAg+/HBcAb+	3	0.93%
Total	321	100%

3.2 Distribution of HBV-DNA loads

According to the distribution of HBV-DNA loads, there were 244 cases with low load, 61 cases with medium load and 16 cases with high load. In the low load group, the number of HBsAg+/HBeAb+/HBcAb+ cases was the highest at 198, and the mean value of HBsAg+, HBsAb+, HBeAb+, HBcAb+ was the highest at 1.80×10^4 IU/ml. In the medium load group, the number of HBsAg+, HBeAg+, HBcAb+ cases and the mean value were all the highest at 32 and 2.95×10^7 IU/ml, respectively. In the high load group, the number of HBsAg+, HBeAg+, HBcAb+ cases and the mean value were also the highest at 15 and 2.12×10^8 IU/ml. The distribution of data is shown in Table 2.

Table 2: Distribution of HBV-DNA load in different serologic patterns (n=321)

Serologic marker	Low load ($<1.00\times10^5$IU/ml)		Medium load (1.00×10^5~1.00×10^8IU/ml)		High load ($\geq1.00\times10^8$IU/ml)	
	Cases	Mean value	Cases	Mean value	Cases	Mean value
HBsAg+/HBeAg+/HBcAb+	32	8.08×10^3	32	2.95×10^7	15	2.12×10^8
HBsAg+/HBeAb+/HBcAb+	198	7.20×10^3	22	4.03×10^6	0	/
HBsAg+/HBeAg+/HBeAb+/HBcAb+	4	1.55×10^4	3	5.31×10^5	0	/
HBsAg+/HBsAb+/HBeAg+/HBcAb+	3	6.29×10^3	3	2.20×10^6	1	1.41×10^8
HBsAg+/HBsAb+/HBeAb+/HBcAb+	5	1.80×10^4	0	/	0	/
HBsAg+/HBcAb+	2	3.41×10^3	1	1.35×10^5	0	/
Total	244	7.63×10^3	61	1.70×10^7	16	1.98×10^8

3.3 HBV-DNA distribution in HBeAg-positive and HBeAb-positive patients

Comparison of HBeAg-positive and HBeAb-positive DNA load distribution showed that HBeAb-positive low load distribution was higher than that of HBeAg-positive, while medium-high load distribution was lower in HBeAb-positive than in HBeAg-positive, with a significant difference for the variance analysis results ($P<0.05$), as shown in Table 3.

Table 3: DNA load distribution of HBeAg-positive and HBeAb-positive patients

Serologic marker	Low load	Medium load	High load	Total
HBeAg+	35a	35	16a	86
HBeAb+	203	22	0	225
HBeAg+/HBeAb+	4	3	0	7

Note: aP<0.05 indicates a statistically significant result compared to HBeAb-positive samples.

4. Discussion

Hepatitis B, a contagious liver disease caused by HBV is a global public health problem. Hepatitis B virus infection is very common all over the world, especially in Asia and Africa [6]. Continuous development of hepatitis B can lead to adverse consequences like liver cancer or cirrhosis, posing a great threat to the normal life and health of patients. Therefore, early diagnosis of hepatitis B is important for the improvement of patients' conditions [7]. At this stage, the collection of serum for laboratory testing has become an important means of diagnosis of hepatitis B. The detection of HBV-DNA load and hepatitis B-related antibody and antigen indicators is possible to understand the HBV infection status of the physical examinees in a timely manner, providing a reasonable diagnostic reference and a favorable help for further diagnostic and therapeutic work.

Real-Time PCR and CLEIA are both effective methods for detecting HBV infection, but there are differences in the principles. Real-Time PCR is a nucleic acid assay used to detect and quantify specific DNA or RNA sequences, but it is specifically used to quantify viral load in the case of HBV testing, that is, the number of viruses in the body [8]. During PCR, a fluorescently labeled probe is used to bind to amplified DNA fragments to perform real-time monitoring by changes in the fluorescence signal, with the fluorescence signal intensity being directly proportional to the amount of viral DNA in the initial sample [9]. CLIA is an antibody-antigen reaction-based assay used to detect hepatitis B markers in the blood, such as HBsAg, HBsAb, HBcAb, HBeAg, and HBeAg. During the detection, the sample was first added to a reagent containing one of the antibodies with high affinity for the HBV antigens or antibodies, with antigens or antibodies to HBV in the sample binding to the reagent antibodies or antigens [10]. Then, an enzyme-labeled

secondary antibody was usually added and a substrate was added to trigger a chemiluminescent reaction, with luminescence signal intensity being proportional to the amount of the specific marker in the sample. Finally, a luminescence detector was used to measure the light signal emitted to qualitatively or quantitatively analyze the level of HBV markers in the sample. The advantage of Real-Time PCR is that it can provide quantitative information, whereas the advantage of CLIA is that it is suitable for large-scale screening for HBV markers with its high sensitivity and specificity [11].

Hepatitis B virus surface antigen reflects early hepatitis B virus infection and is one of the important indicators of serological detection. In the study, the highest positivity rates were found for HBsAg+, HBeAb+ and HBcAb+, followed by HBsAg+, HBeAg+ and HBcAb+. Further analysis of the distribution of HBV-DNA copies showed that HBsAg+, HBeAb+, HBcAb+ accounted for 198 of the 244 low-load samples, indicating that their DNA loads were generally low. Among the 61 medium-load samples and 16 high-load samples, HBsAg+, HBeAg+ and HBcAb+ samples accounted for 32 and 15 cases, respectively, indicating that their viral loads were high. The detection of HBeAg positivity or elevated HBeAg in patients indicated that the hepatitis B virus was actively replicating when the patients were strongly infectious and had a high copy number of HBV-DNA. The appearance of HBeAb indicated that the replication of the virus in the body was weakened, the copy number decreased, and the infectiousness of the virus also decreased gradually. In the study, 7 samples were positive for both HBeAb and HBeAg, which may be due to that two markers in the patient's body were in a dynamic equilibrium of the conversion [12]. During the progression of chronic hepatitis B, there is a process of serological conversion, which should be the disappearance of HBeAg and the emergence of HBeAb, which may be positive for some patients, indicating an increase in the replication of the hepatitis B virus in the patient [13].

The above results showed that there is a good correlation between the HBV-DNA load level detected by Real-Time PCR and hepatitis B half-and-half detected by CLIA. The main reason is that Real-Time PCR directly measures the number of viral DNA copies, providing high sensitivity even when the viral level is low. In contrast, CLIA detects the immunologic markers associated with viral infection, with positive results influenced by the individual immune response strength. Differences in the number of DNA copies in different combinations of positives indicate that the actual viral load varies between infected individuals [14]. The information is important for assessing the infection severity, therapeutic effect, and the risk of chronic infection. It was also found that the distribution of HBeAb-positive low loads was higher than that of HBeAg-positive, while the distribution of medium and high loads was statistically higher in HBeAg-positive than in HBeAb-positive (P<0.05).

However, it should be noted that the actual testing of Real-Time PCR should note the following points even with a high positivity rate. First, it is important to ensure that the samples are not contaminated during sample collection, storage, and transportation during sample processing, which is essential to ensure the accuracy of the test results. In addition, measures should be taken to prevent DNA degradation in blood samples. Second, it is important to follow strict protocols to avoid cross-contamination between samples when handling samples in the laboratory, especially during the PCR amplification phase. Third, the efficiency of the PCR reaction affects the final quantitative result and it is important to ensure that the amplification efficiency is within reasonable limits. False-positive results can be caused by environmental contamination, reagent contamination, or equipment contamination, which can be avoided by appropriate measures. As false negatives can be caused by undetectable viral loads or operational errors, it is important to ensure that a sufficiently sensitive RT-PCR protocol is used.

5. Conclusion

In summary, Real-Time PCR and CLIA can achieve high value in practical applications for the detection of HBV infection and can reflect the corresponding viral antigen or antibody positivity, with a good correlation. The combined application of the two methods can identify the lesions at an earlier stage and fully reflect the disease outcome and the prognosis, providing a favorable reference for clinical diagnosis and treatment, as well as preventing the continuous progress of the disease. Therefore, it is worthwhile to popularize the combined application of Real-Time PCR and CLIA.

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