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ORIGINAL ARTICLE

Detection of HBV DNA by PCR and its application in clinical transfusion

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Abstract: This study was done in order to detect the hepatitis B virus (HBV) DNA copies in patients through blood transfusions; recessive carriers with HBsAg negative but HBV DNA positive were further studied to see the content and distribution of HBV in patients, and provide evidence for the clinical treatment. A total of 532 blood samples collected from July 2014 to July 2015 were tested for HBV-DNA viral load and hepatitis B serological markers using quantitative Polymerase Chain Reaction (qPCR) and serologic test (the five serological markers of hepatitis B). The results showed that, 3 cases were HBV serology negative and the HBV-DNA viral load was in the range of 250–500 whereas only 1 case was HBsAb positive and the HBV-DNA viral load was in the range of 500 and above. Quantitative PCR, for detecting HBV DNA, together with serological routine test can effectively reduce the risk of transfusion and prevent medical disputes.

Keywords: Hepatitis B virus, HBsAg; Serological markers; Screening

Introduction

Hepatitis B virus (HBV) is highly contagious. It can cause chronic infection and puts people at high risk of death from cirrhosis, portal hypertension, and liver cancer. Hepatitis B virus belongs to the family hepadnaviruses (hepatotropic DNA virus); whereby humans are the only natural host. Hepatitis B virus enters the liver through bloodstream and replicates within the infected liver cells[1].

Patients with occult hepatitis B showed hepatitis B surface antigen (HBsAg) negative, but present as circulating HBV DNA in serum or liver tissue. These patients may not have the hepatitis B virus antibody (HbsAb). Although there is unclear understanding on this, but research indicates that occult HBV infection is one of the risk factors leading to liver cancer[2].

HBV is transmitted through blood transfusion; the rate of transfusion-transmitted HBV infections were varies in different regions. According to an epidemiological survey, the rate of transfusion-transmitted HBV infections in 11 major cities in Indonesia is about 2.1% to 9.5% or even up to 10.5% in Papua[3].

doi: 10.18686/aem.v5i1.69

Materials and methods

Specimen sources

A total of 532 blood samples were collected in our hospital from July 1, 2014 to June 31, 2015. Approximately 4 mL of blood was withdrawn from fasting patient and stored at room temperature for 30 to 60 min, whereby the serum was separated by cryopreservation at -20 ℃ for 2 h to measure HBV-DNA, while the other tube was screened using enzyme-linked immunosorbent assay (ELISA) method with Hepatitis B serum indicators.

Main instruments

The main instruments used in the study were namely, ABI Prism 7500 Real-Time PCR system, automatic microplate washer, microplate reader, and autosampler.

Sample detection

HBV immunoassay kit was purchased from InTec Ltd. ELISA method was used to detect anti-HCV antibodies in serum, with the main wavelength was set at 450 nm, while the sub-wavelength was at 630 nm. Optical Density (OD) value of each reading was measured. HBV DNA Fluorescence PCR detection method using HBV DNA standard kit was provided by Guangzhou Da An Gene Co, Ltd. with HBV genomes targeted the conserved regions using an internal standard to monitor the entire process. Handling of test specimens and quality control samples was carried out according to quantitative reference materials, with the presence of positive and negative controls. HBV DNA was extracted from 100 μL of lysis boiled serum with a final volume of 25:1 (v/v), of which, 2 μL of supernatant was used as template in PCR amplification system, with the mixture constituents of 2 μL template and 38 μL fluorescent PCR reagents. The test was done using FQ-PCR amplification ABI Prism 7500 with the following conditions: 10 cycles of 93 ℃ for 2 min, 93 ℃ for 45 s, and 55 ℃ for 60 s; then, 30 cycles of 93 ℃ for 30 s and 55 ℃ for 45 s, while the set-up for thresholds and baselines was referred to the kit's instructions. The study also followed the requirements of the ISO 15189 Medical Laboratory by quantitatively comparing the old and new reagents (EP9-A).

Statistical analysis

Statistical analysis was done using SPSS 18.0 statistics software, all p-value which are less than 0.05 were considered significant.

Results

General situation

From July 2014 through July 2015, a total of 532 cases were detected as HBV DNA positive in 294 men (55.3%), with the mean age of 47.7 years, and in 238 women (44.7 %), with the mean age of 42.9 years. The age group is divided into 2 groups; 16 to 45 years and 46 to 85 years, with the percentage for each category were 60% and 40%, respectively (*Table 1*).

Table 1 Basic information for detection of HBV DNA in patients

Age (Years)	Male (N = 294)	55.3%	Female (N = 238)	44.7%	Total (N = 532)	(100%)
16 - 45	197	37.0%	122	22.9%	319	60.0%
46 - 85	97	18.3%	116	21.8%	213	40.0%

Correlation between HBV serological test and detection of HBV DNA

Table 2 shows that, a total of 82 cases of HBV DNA copy number of more than 500 was detected (15.4% of the total amount) while 11 cases were found to have 251−500 of HBV DNA copy number (4.7% of the total amount). On the

other hand, 177 cases were serological detected as HBsAg positive (33.3%); 195 cases were HBsAb positive (36.7%); and 271 cases were HBcAb positive (50.9%), of which, 81 out of 82 cases were having HBV DNA copy number > 500 and HBcAb positive (98.8%). The chi-square test shows both consistencies of the data ($p < 0.05$).

Table 2. Correlation between HBV serological test and detection of HBV DNA

	HBV DNA copy number					
HBV serological test	0–1	2–50	51–150	151–250	251–500	>500
All negative	41	64	4	0	3	0
HBsAb	56	86	4	2	0	1
HBcAb	6	12	0	0	0	0
HBsAg/ HBcAb	1	5	2	0	0	2
HBsAb/ HBcAb	10	21	1	1	0	0
HBeAb / HBcAb	7	7	0	0	0	1
HBsAg/HBeAg/ HBcAb	0	1	0	0	0	11
HBsAg/HBeAb/ HBcAb	10	44	15	12	7	67
HBsAb/HBeAb/ HBcAb	8	14	1	0	0	0
Unknown	2	2	0	0	1	0

Discussion

Due to the standard use of reliable serological screening before transfusion, hepatitis caused by blood transfusion has been extremely rare, but still unavoidable. Screening of blood for HBV surface antigen (HBsAg) as diagnostic's criteria is still incomplete in eliminating the risk of transfusion-transmitted HBV. Our study showed that HBV DNA was serological detected in a patient with negative HBV but positive for HBsAg. HBV DNA, however, still can be detected in some HBsAg-negative blood samples[4]. This is probably related to HBsAg seronegative window period, in which, during this window period, the viral replication was slow. A person with weakened immune system will not trigger immune response against virus immediately and no clinical symptoms were manifested during the early stage of infection. The symptoms occur after a period of blood transfusion, resulting in medical disputes[5]. Viral mutation and atypical seroconversion may also cause the result unreliable. Therefore, a more sensitive techniques and high accuracy test such as PCR, for detecting HBV DNA, is necessary to use in screening blood transfusions.

Conclusion

In summary, the serological routine test has several limitations and could easily lead to missed diagnosis or misdiagnosis. There are still doubts if using quantitative PCR method alone in blood screening. Therefore, examination before transfusion using quantitative PCR for detecting HBV DNA together with serological routine test can effectively reduce the risk of transfusion and prevent medical disputes.

Conflict of interest

The author declares no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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