



Case report

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

Shunqing Li

Correspondence: 2822417@qq.com

Laboratory of Hubei Provincial Hospital of Integrated Chinese and Western Medicine, Wuhan, Hubei Province, China

Abstract: This study was 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 (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 above 500. qPCR, for detecting HBV DNA, together with serological routine test can effectively reduce HBV infection during transfusion and prevent medical disputes.

Keywords: Hepatitis B virus, HBsAg, serological markers, screening

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Introduction

Hepatitis B virus (HBV), a member of the the family hepadnaviruses (hepatotropic DNA virus), is highly contagious. Humans are its sole natural host. It enters the liver through bloodstream and proliferates within the infected liver cells^[1], then causes chronic infection and puts people at high risk of death from cirrhosis, portal hypertension, and liver cancer.

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

HBV can be transmitted through blood transfusion, and the incidence of transfusion-transmitted HBV infections varies in different regions. According to an epidemiological survey, it was 2.1-9.5% in 11 major cities of Indonesia and even up to 10.5% in Papua^[3].

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 Approximately 4 mL of blood was withdrawn from a fasting patient and stored at room temperature for 30 to 60 min, whereby the serum was separated by cryopreservation at -20 °C for 2 h to measure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 was used to detect anti-HCV antibodies in serum. The main wavelength was at 450 nm and the sub-wavelength was at 630 nm. Optical Density (OD) value of each reading was measured. Fluorescence qPCR kit was purchased from Guangzhou Da-An Gene Co, Ltd., which targeted the conserved regions of the HBV genome using an internal standard to monitor the entire process. Handling of test specimens and quality control was carried out according to quantitative reference materials, with the presence of positive and negative controls. HBV DNA was extracted from 100 μ L of lysis boiled serum with a final volume of 25:1 (v/v). 2 μ L of supernatant was used as template in PCR, mixed with 38 μ L fluorescent PCR reagents. The test was done using FQ-PCR amplification ABI Prism 7500 with the following conditions: 10 cycles of 93°C for 2 min, 93°C for 45 s, and 55°C for 60s; 30 cycles of 93°C for 30 s and 55°C for 45 s. The set-up of thresholds and baselines was referred to the manufacture's instructions. The study was in compliance with the ISO 15189 Medical Laboratory by quantitatively comparing the old and new reagents (EP9-A).

Statistical analysis

Statistical analysis was done using SPSS v18.0. $P < 0.05$ was considered a significant difference.

Results

General situation

From July 2014 to July 2015, a total of 532 cases were identified HBV DNA positive in 294 male (55.3%), with a mean age of 47.7 years, and in 238 female (44.7 %), with a mean age of 42.9 years. The young group included cases between 16-45 year-old, and the old group included cases between 46 to 85 years, respectively (*Table 1*).

Table 1 Basic information of the subject 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 (15.4% of the total amount) had a HBV DNA copy number of more than 500, while 11 cases (4.7%) had 251-500 copies. 177 cases (33.3%) were serologically HBsAg positive, 195 cases (36.7%) were HBsAb positive, and 271 cases (50.9%) were HBcAb positive. 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 serological test	HBV DNA copy number					
	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 serologically 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 due to the HBsAg seronegative window period, during which the virus amount is too slow to trigger the immune response, so that no clinical symptoms could be observed. Symptoms occur after a period of blood transfusion, resulting in medical disputes^[5]. Viral mutation and atypical seroconversion may also render the result unreliable. Therefore, a more sensitive technique and high accuracy test such as PCR, for detecting HBV DNA, are in demand.

Conclusion

In summary, the serological routine test has several limitations and could easily lead to misdiagnosis. There are still doubts that using qPCR alone in blood screening is sufficient. Therefore, examination before transfusion using qPCR to detect HBV DNA together with serological routine test can effectively reduce the risk of viral infection during blood 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.

Reference

1. Rawat S, Sharma N, Kumar M, Ahmed NK, Sailwal S, et al. Quantification of HBV DNA and its impact on viral load monitoring. *JBPRAU* 2013; 2(4): 75–80.
2. Wong DKH, Huang FY, Lai CL, Poon RTP, Seto WK, et al. Occult hepatitis B infection and HBV replicative activity in patients with cryptogenic cause of hepatocellular carcinoma. *Hepatology* 2011; 54(3): 829–836. doi: 10.1002/hep.24551.
3. Lusida MI, Nugrahaputra VE, Soetjipto, Handajani R, Nagano-Fujii M, et al. Novel subgenotypes of hepatitis B virus genotypes C and D in Papua, Indonesia. *J Clin Microbiol* 2008; 46(7): 2160–2166. doi: 10.1128/JCM.01681–07.
4. Seo DH, Whang DH, Song EY, Kim HS, Park Q. Prevalence of antibodies to hepatitis B core antigen and occult hepatitis B virus infection in Korean blood donors. *Transfus* 2011; 51(8): 1840–1846. doi: 10.1111/j.1537–2995.2010.03056.x.
5. Huang J, Liu S, Zhang J, Peng L, Li Q, et al. Blood HBV, HCV and HIV nucleic acid detection in clinical transfusion: Application of NAT testing for HBV, HCV and HIV in clinical blood transfusion. *Chin J Clin Hematol* 2013; (1): 95–97.