Relationship between serum Hepatitis B virus (HBV) DNA levels and HBeAg status in patients with Hepatitis B virus infection

Vandana Kaushal, Veenu Gupta, Deepinder Chhina

Department of Microbiology,
Dayanand Medical College & Hospital, Ludhiana, India

ABSTRACT

Background*:* Hepatitis B is one of the most common types of viral hepatitis in the world. For the last thirty years, only serological markers and liver function test have been utilized to monitor the disease progression and treatment response until the emergence of molecular detection methods. Hepatitis B V DNA quantitation is used extensively world wide for the diagnosis and monitoring of treatment of Hepatitis B virus (HBV) infection. The **aim** of this study was to quantitate HBV–DNA by Real time PCR method and to compare the results with HBeAg detection in Hepatitis B patients.

Material and Methods*:* Seventy one serum samples of patients with hepatitis (all HBsAg positive) were the subjects of this study. Serum HBV DNA of all these samples was detected by COBAS TaqMan real time PCR and HBeAg by ELISA.

Results: Amongst Hepatitis B group patients, serum HBV DNA was detected in 61 out of 71 patients (85.9%). HBeAg was positive in 21% of patients (15/71). Majority of the HBeAg positive patients had a significantly higher serum HBV DNA levels than HBeAg negative patients.

Conclusion: HBeAg status did not necessarily reflect HBV-DNA level in the serum, as 46/71 (64.7%) in the Hepatitis B group were positive for HBV DNA but negative for HBeAg.

Keywords: Hepatitis B, HBV–DNA, HBeAg, Real time-PCR

INTRODUCTION

Approximately one third of world population has serological evidence of past or present hepatitis B virus (HBV) infection resulting in 400 million chronically infected people.[1] This has led a variety of clinical outcomes ranging from apparently healthy asymptomatic carrier state to acute or chronic liver disease including cirrhosis and hepatocellular carcinoma.[1,2] Persistent presence of Hepatitis B surface antigen for at least six months defines the chronic hepatitis B (CHB) carrier state.[3] The presence of hepatitis B surface antigen (HBsAg) in serum indicates HBV infection, but does not provide information on the replicative state of the virus. Hepatitis B e antigen (HBeAg) has been considered a viral replicative marker.[3, 4] Whether spontaneous or due to therapy, HBeAg seroconversion is not only an important transition point throughout the natural course of chronic HBV infection but is also a significant factor in determining the clinical course of the disease.[5,6] Hepatitis B V-DNA in serum has become an important tool to identify individuals with high viral replication, to monitor patients on therapy, and to predict whether antiviral therapy will be successful.[7,8]

The aim of the study was to quantitate Hepatitis B V-DNA by real time PCR method and to compare the results of HBV-DNA estimation with HBeAg in Hepatitis B patients.

MATERIAL AND METHODS

71 serum samples were collected from hepatitis B group of patients over a period of six months (January –June 2011) in a tertiary care hospital in North India. All these patients were HBs Ag positive. Hepatitis B V DNA of all these samples was extracted from 500µl of serum by high pure viral nucleic acid kit according to manufacturer's

Corresponding Author :

Dr Deepinder Chhina
Head, Department of Microbiology,
Dayanand Medical College and Hospital,
Ludhiana-141001
E-mail: deepinder.chhina@rediffmail.com

instructions. The COBAS TaqMan 48 analyzer was used for automated real time PCR amplification and detection of PCR products according to the manufacturer's instructions. The data thus generated was analysed with Amplilink software. HBV DNA levels were expressed in IU/ml (Lower limit of detection: 6 IU/ml). HBeAg levels were determined using commercially available ELISA (Mfd German Biologicals Corpo, Taiwan) and the subjects were divided into two groups based on hepatitis e antigen (HBeAg) status. Group I patients were HBeAg positive, while group II were HBeAg negative. Results were considered statistically significant at $P < 0.05$.

RESULTS

Out of these 71 patients, 52 were males and 19 were females. They were in the age group of 16-77 years. Serum HBV DNA was detected in 61 out of 71 patients (85.9 %) subjected to real time PCR. HBeAg was positive in 21% of patients (15/71).Out of these 15 were positive by both HBV DNA and HBeAg, whereas 10 were negative by both the tests. Forty six patients were positive by real time PCR for HBV DNA but were negative for HBeAg (Table -1). Serum HBV DNA was detected in all the patients who were HBeAg positive and 46 out of 56(82.1%) patients who were negative for HBeAg. Group I had 15 patients where as 56 patients belonged to Group II. Majority of the patients in Group I had a significantly higher serum HBV DNA levels than Group II patients (Figure -1). The HBV viral load in Group I was in the range of $1.14x10^5$ – $>1.1x10^8$ IU/ml (Mean $\log_{10}$ HBV DNA = 6.8±1.9) which was statistically very significant from the viral load in Group II that ranged between <6 to $>1.1x10^8$ IU/ml (Mean $\log_{10}$ HBV DNA= 3.9±2.4) P value 0.000.

Table 1

Status of HBV DNA and HBeAg in hepatitis B infection cases (n=71)

Tests	Number (%)
Positive by both HBV DNA and HBeAg	15(21.1)
Negative by both HBV DNA and HBeAg	10 (14)
Positive by HBV DNA only	46(64.7)

DISCUSSION

The quantitative real time PCR assay for the detection of HBV-DNA is a highly sensitive method. Measurement of serum HBV-DNA concentrations in Hepatitis B patients facilitates prediction of hepatic inflammatory activity. In the present study HBV-DNA was detected in the serum of 85.9% (61/71) whereas HBeAg was positive in 21% (15/71) patients of Hepatits B group. Majority of the patients in Group I (HBeAg positive) had a significantly higher serum HBV DNA levels than Group II (HBeAg negative) patients similar to that reported in literature .[9,10] HBeAg status did not necessarily reflect HBV-DNA level in the serum, as 46/71 (64.7%) were

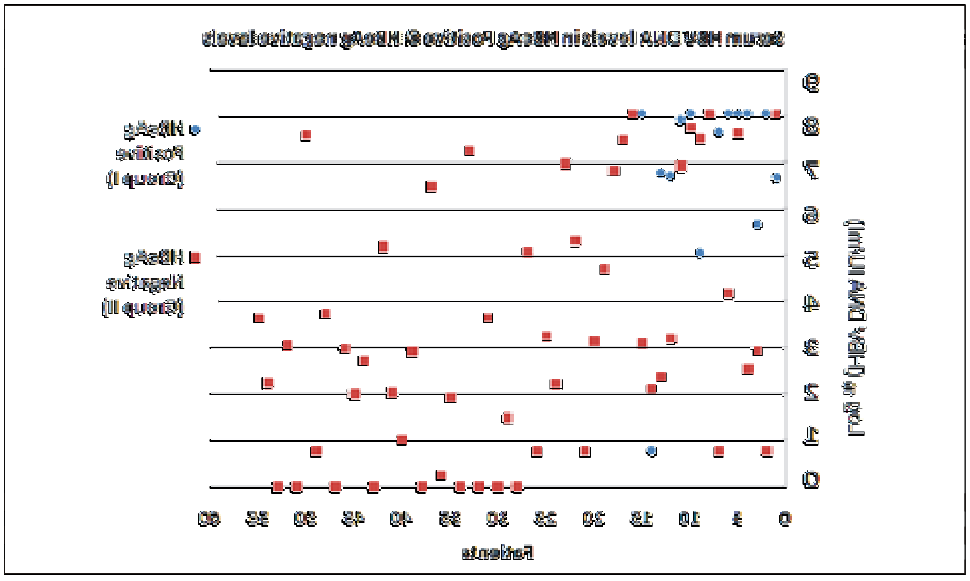

Fig. 1

positive for HBV DNA but negative for HBeAg, which is in agreement with previous studies. [11,12] Thus, it can be infered that the real time PCR method of detection of HBV – DNA is better for the monitoring and management of Hepatitis B infection as the traditional method of detection of HBeAg can miss some cases of Hepatitis B. To conclude, management of chronic hepatitis B carriers is no longer dependent on the hepatitis e antigen status and liver function tests. Though they can be used in conjunction with the serum HBV DNA levels.

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