

## Original Research Article

# Correlation of HBsAg quantitation by ELISA with serum hepatitis B virus DNA quantitative PCR in chronic hepatitis B patients

Sandesh Kolassery\*

Department of Medical Gastroenterology, Government Medical College, Kozhikode, Kerala, India

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**\*Correspondence:**

Dr. Sandesh Kolassery,

E-mail: [drsandeskh@gmail.com](mailto:drsandeskh@gmail.com)

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## ABSTRACT

**Background:** Serum HBV DNA is a useful and reliable marker to diagnose and monitor CHB on treatment. The limitation of HBV DNA is that it is expensive and that the assays lack uniformity and standardization. Hence there is a need for more economical and reliable marker. HBsAg quantitation is one such surrogate serological marker. The objective of the current study is to compare and correlate the serum hepatitis B DNA quantitative PCR with HBsAg quantitation.

**Methods:** Patients with CHB attending to the outpatient clinic of Gastroenterology department were enrolled in the study. Patients with undetectable HBV DNA levels and those co-infected with HCV or HIV were excluded from the study. All patients were tested for serological markers like HBsAg (rapid), HBeAg, anti HBe and HBV DNA-PCR. HBsAg quantification was done using conventional ELISA immunoassay. HBV-DNA and qHBsAg levels were expressed in log<sub>10</sub> IU/ml. Pearson correlation was used to estimate correlation between HBV DNA and HBsAg quantitation. Statistical analysis was done using SPSS and P value of <0.05 was considered significant.

**Results:** A total of 38 patients were enrolled in the study. 23.62% were females and mean age of patients in the entire study group was 35.72 years. The mean ALT level was 103.80 U/L. 26.32% (n = 10) were HBeAg positive. Mean HBV DNA and qHBsAg levels for the entire cohort were 5.81 log<sub>10</sub> IU/ml and 5.83 log<sub>10</sub> IU/ml respectively with a correlation coefficient of 0.318 (P = 0.130). For HBeAg positive patients the mean HBV DNA and qHBsAg levels were 7.90 log<sub>10</sub> IU/ml and 5.91 log<sub>10</sub> IU/ml respectively with a correlation coefficient of 0.722 (P = 0.043). HBV DNA levels were significantly higher in HBeAg positive patients compared with HBeAg negative patients (7.9 versus 4.01; P = 0.002). qHBsAg levels were also marginally high in HBeAg positive patients (5.91 versus 5.8; P = 0.136). Neither HBV DNA levels nor qHBsAg levels correlated with serum ALT levels.

**Conclusions:** There is a significant correlation between quantitative HBsAg levels and HBV-DNA levels in HBeAg positive patients with chronic hepatitis B but not in HBeAg negative patients. HBV-DNA levels are significantly higher in HBeAg positive patients.

**Keywords:** Chronic hepatitis B, HBsAg quantitation, Hepatitis B DNA PCR, Quantitative HBsAg

## INTRODUCTION

An estimated 400 million persons in the world today are chronically infected with HBV. The majority of these individuals will not experience complications, but 15% to 40% will have serious sequelae such as cirrhosis or

hepatocellular carcinoma (HCC), and many will die prematurely.<sup>1,2</sup> The prevalence of HBV infection varies markedly around the world. In highly endemic regions, such as Southeast Asia (excluding Japan), China, and much of Africa, 8% or more of the population are chronic HBV carriers, and the lifetime risk of infection ranges from 60% to 80%.<sup>3</sup> Hepatitis B virus (HBV) infection

continues to remain a significant global health problem. Estimates of the World Health Organization (WHO) suggest that more than 2 billion people worldwide have been infected with HBV. Of these, approximately 240 million individuals have chronic (long-term) liver infections and at risk of serious illness and death, mainly from liver cirrhosis and hepatocellular carcinoma (HCC). More than 780 000 people die every year due to the acute or chronic consequences of hepatitis B.<sup>4-7</sup>

Periodic serum HBV DNA is the most reliable marker to monitor CHB patients on treatment.<sup>8,9</sup> However, serum HBV DNA measurement has several limitations. Despite being expensive, labour-intensive HBV DNA assays lack uniformity and standardization. Many commercial kits are available and all have different linear ranges, lower limits of detection and conversion factors. Repeated assays done preferably on the same platform are needed to monitor a patient on antivirals. Hence, there is a definite need of a monitoring tool which is economical, reliable and easy to perform. HBsAg quantitation is a recent serological marker being evaluated. Though it is not new, a fully automated version of this assay has recently been introduced.<sup>10</sup> This method is based on ELISA Immunoassay and results are expressed as IU/ml.

Since its description was made, various studies have come up where clinical utility of HBsAg quantitation has been described, but studies where these two markers have been compared are scarce and conflicting results are available.<sup>11-16</sup> There are no data from the Indian subcontinent where these two markers have ever been evaluated. Therefore, the present study was undertaken to correlate HBV DNA and HBsAg levels in CHB patients and also to find out the subgroup of patients where it can be more suitably used.

## METHODS

The study was carried out from June 2013 to November 2013. All consecutive patients of CHB attending the out-patient clinic were included. Following patients were excluded:

- Patients with undetectable HBV DNA levels
- Patients with co-infection with HCV, human immunodeficiency virus (HIV) or hepatitis D virus (HDV);
- Patients with autoimmune liver disease
- Patients who did not give consent.

### Clinical evaluation

Complete clinical histories of all the patients were taken. All study subjects' sera were tested for routine hepatitis B serological markers HBsAg, HBeAg. All sera were subjected to HBV DNA by real-time polymerase chain reaction (PCR) and in all of them HBsAg quantification was done by ELISA Immunoassay by Alfa Diagnostics International.

### HBV DNA quantitation

HBV DNA quantitation was done on patient's plasma using COBAS TaqMan HBV test with high pure extraction (Roche Diagnostics) as per the manufacturer's protocol. This is a real-time PCR assay. Results were expressed as IU/ml.

### HBsAg quantitation

HBsAg levels were measured by the fully automated Architect HBsAg QT (Alfa Diagnostic International) assay as per the manufacturer's protocol and the results were expressed as IU/ml. This assay is calibrated against the WHO standard and allows the quantitation of HBsAg from 0.05 to 250 IU/ml. A concentration higher than 0.05 IU/ml was considered HBsAg positive. Samples with an HBsAg level higher than 250 IU/ml required a 1:500 dilution with the diluent as recommended by the manufacturer and the exact concentration of HBsAg was measured.

### Statistical analysis

Quantitative variables were expressed as median with range and qualitative variables were expressed as numbers with percentage. Pearson correlation was used to estimate correlation between HBV DNA and HBsAg quantitation. Statistical analysis was done using SPSS and p value of <0.05 was considered significant.

## RESULTS

A total of 38 patients were enrolled in the study. 23.62% were females and mean age of patients in the entire study group was 35.72 years.

**Table 1: Baseline variables.**

Baseline variables	
Mean age	35.72±14.73
Males %	76.8%
HBeAg positive %	26.32%
ALT U/L	103.80±223.6
HBV DNA (log <sub>10</sub> IU/ml)	5.81±2.46

The mean ALT level was 103.80U/L. 26.32% (n=10) were HBeAg positive. Mean HBV DNA and qHBsAg levels for the entire cohort were 5.81 log<sub>10</sub>IU/ml and 5.83 log<sub>10</sub>IU/ml respectively with a correlation coefficient of 0.318 (p=0.130). For HBeAg positive patients the mean HBV DNA and qHBsAg levels were 7.90 log<sub>10</sub>IU/ml and 5.91 log<sub>10</sub>IU/ml respectively with a correlation coefficient of 0.722 (p=0.043). HBV DNA levels were significantly higher in HBeAg positive patients compared with HBeAg negative patients (7.9 versus 4.01; p=0.002). qHBsAg levels were also marginally high in HBeAg positive patients (5.91 versus

5.8;  $p=0.136$ ). Neither HBV DNA levels nor qHBsAg levels correlated with serum ALT levels.

**Table 2: Subgroup analysis.**

Subgroup analysis		
HBV DNA (log <sub>10</sub> IU/ml)		
HBeAg positive	7.90	P=0.002
HBeAg negative	4.01	
qHBsAg (log <sub>10</sub> IU/ml)		
HBeAg positive	5.91	P=0.136
HBeAg negative	5.80	

**Table 3: Different group variables.**

Variables	Correlation	P Value
Whole study group		
HBV DNA versus qHBsAg	0.318	0.130
HBV DNA versus ALT	0.088	0.696
qHBsAg versus ALT	0.157	0.426
HBeAg positive		
HBV DNA versus qHBsAg	0.722	0.043
HBV DNA versus ALT	0.105	0.822
qHBsAg versus ALT	0.291	0.447
HBeAg negative		
HBV DNA versus qHBsAg	0.142	0.660
HBV DNA versus ALT	-0.297	0.375
qHBsAg versus ALT	-0.106	0.707

## DISCUSSION

CHB infection is a serious clinical problem with a worldwide distribution and has adverse sequelae. In Asia and India, majority of HBV infection is acquired perinatally or in early childhood. The prevalence of CHB in India is in the intermediate range with an estimated 40 million subjects infected.<sup>17</sup> Long term suppression of viral replication is required for reducing the complications of CHB infection. Chronic hepatitis B patients require long-term monitoring during treatment. Methods of monitoring treatment response include LFT, HBV DNA, HBeAg, anti-HBe, HBsAg, and liver histology.<sup>18</sup>

Quantitative serology can be done by HBsAg. HBsAg is encoded by the envelope gene, which contains three open-reading frames: the pre-S1, pre-S2 and S domains. There is subsequent conversion to small, medium and large forms of HBsAg proteins. Newly synthesised HBsAg proteins are secreted from the hepatocyte. Similar to the HBeAg pathway, HBsAg synthesis is separate from the viral replication pathway. Existent quantitative HBsAg serology can detect all three forms of HBsAg in the circulation. Since the introduction of quantitative HBsAg, a lot of studies have come up regarding its clinical significance.<sup>19</sup> Many cross-sectional studies have shown significant correlation between HBsAg levels and serum HBV DNA levels.<sup>20-22</sup> Negative or poor correlation

between these two markers has also been studied by many researchers.<sup>19,23</sup>

## CONCLUSION

This study shows that there is a significant correlation between quantitative HBsAg levels and HBV-DNA levels in HBeAg positive patients with chronic hepatitis B but not in HBeAg negative patients. HBV-DNA levels are significantly higher in HBeAg positive patients. The limitation of the study is the small number of patients included in the study. Larger studies are required to confirm that HBsAg quantitation is comparable to hepatitis B DNA PCR.

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