Real Time PCR HBV-DNA Analysis in HBsAg Positive Patients

Rahman W¹, Hossain MR², Khan AA³, Saha D⁴, Alam SMM⁵, Yasmin F⁶

Abstract

Introduction: The hepatitis B virus is a global public health concern and leading cause of chronic liver disease in Bangladesh. For the diagnosis and monitoring of treatment of Hepatitis B virus infection, HBV-DNA detection and quantification is now extensively used worldwide.

Objectives: The objective of this study was to detect HBV-DNA by real time PCR method in HBsAg positive patients, to compare the results of HBV-DNA detection with HBeAg and Anti-HBe and to monitor the response after antiviral therapy in chronic hepatitis B patients and also to observe the intensity of hepatitis B infection in relation to age and sex.

Methods: This was a cross sectional type of study conducted in Armed Forces Institute of Pathology (AFIP), Dhaka Cantonment. In this study, 56 sera of HBsAg positive patients were selected who all were subjected to do HBV-DNA (real time PCR) analysis during the period of 29 July to 30 October, 2013.

Results: Out of 56 HBsAg positive patients, HBV-DNA was detected in 34 patients. Among these, 8 (23.5%) patients were HBeAg positive, 16 (47%) patients were anti-HBe positive and 10 (29.5%) were negative for both HBeAg and anti-HBe. Age limit of patients was up to 60 years. HBV-DNA positive patients showed male predominance; 26 (76.5%) patients were male and 8 (23.5%) patients were female. Mean age of the patients was 35±14 years. Among 56 HBsAg positive patients, fifteen were receiving antiviral therapy. Out of them, HBV-DNA was decreased among 4 patients and could not be detected among 11 patients.

Conclusion: Real time PCR method of detection of HBV-DNA is very important in patients who are HBeAg negative and this method is also applied to monitor treatment response to antivirals and to detect occult HBV infections immune control phase and also to detect reactivation of HBV cases.

Key-words: HBV-DNA, real time PCR (rt-PCR), HBeAg, anti-HBe.

Introduction

According to World Health Organization estimates, more than 2000 million people have been infected with HBV worldwide and about 350 million have persistent infection. HBV may be the cause of up to 80% of all cases of hepatocellular carcinoma worldwide, second only to tobacco among known human carcinogens. Approximately, 1 million people around the world die each year from hepatocellular carcinoma or liver failure. Control of HBV infection is therefore, an important goal for public health in areas of endemicity¹⁻⁵. Hepatitis B virus is a double stranded DNA virus of the Hepadnaviridae family of approximately 3,200 base pairs⁶⁻⁸. During the viral replication cycle, supercoiled covalently closed circular viral DNA (cccDNA) is generated and this DNA persists in host cells as a viral minichromosome. This cccDNA can be a source of renewed virus production once the immune response to the acute infection is over and thus constitutes a reservoir of infectious viral particles, thereby leading to the development of chronic hepatitis in certain individuals⁹,¹⁰. HBV-DNA can be detected approximately 21 days before HBsAg typically appears in the serum¹¹. During the window period, when HBsAg

has disappeared and anti-HBs is not yet detectable, HBV-DNA detection can be useful to detect HBV infectious status. Moreover, it is now evident that a group of HBV infected persons may not express HBsAg in the sera due to various factors. Several cases of post-transfusion hepatitis have also occurred after transfusion of HBsAg-negative blood. However, nothing is known about the entity of occult HBV infection or HBsAg-negative HBV-infected subjects in Bangladesh.

Hepatitis B is a complex disease which needs serological, biochemical, molecular & histological evaluation for diagnosis. Recently, the level of HBV-DNA in serum or plasma has been shown to correlate with biochemical and histological measures of disease, and probably reflects more accurately the replicative activity of HBV. Therefore, the measurement of HBV-DNA in serum has become an important tool to identify individuals with high viral replication and to monitor patients on therapy. Various quantitative assays are now used extensively to quantify the level of HBV-DNA load in serum samples. Among them real time PCR is highly sensitive. HBV-DNA persists throughout both acute and chronic disease & can be more accurately detected than HBeAg. It is detectable prior to biochemical evidence of hepatitis.

Materials and methods
This is a retrospective, cross sectional type of study which was conducted in AFIP, Dhaka Cantonment. The study was carried out from 29 July, 2013 to 30 October, 2013. Study populations were HBsAg positive patients of both sex and age limit was up to 60 years. A total of 56 HBsAg positive patients were identified from CMH, Dhaka cantonment, Dhaka. Relevant history was taken from the patients. With the written consent of the patients, firstly, HBV-DNA was detected and quantified by rt-PCR in the molecular biology laboratory, AFIP and secondly, serum HBeAg and anti-HBe were detected by ELISA in the Immunology Department, AFIP, Dhaka Cantonment.

Detection and quantitation of HBV-DNA by rt PCR:
The detection was done by the fluorescent labelling of oligonucleotide probes that bind specifically to the PCR amplicone. Detection of the fluorescence intensity during the course of rt-PCR enables verifi-
Among 34 HBV-DNA positive patients, 8 (23.5%) patients were HBeAg positive, 16 (47%) patients were anti-HBe positive and 10 (29.5%) were negative by both the tests. On the other hand, among 22 patients who were negative for HBV-DNA, none were HBeAg positive, 10 (45.5%) patients were anti-HBe positive and 12 (54.5%) patients were negative for both the tests (HBeAg and anti-HBe) (Table-II).

Table-II: Detection of HBV-DNA by rt-PCR and detection of HBeAg, anti-HBe by ELISA in HBsAg positive patients (n=56).

<table>
<thead>
<tr>
<th>Detection of HBV-DNA</th>
<th>HBeAg positive</th>
<th>Anti-HBe positive</th>
<th>Both HBeAg &amp; anti-HBe negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detected</td>
<td>8 (23.5%)</td>
<td>16 (47%)</td>
<td>10 (29.5%)</td>
</tr>
<tr>
<td>Not Detected</td>
<td>0 (0%)</td>
<td>10 (45.5%)</td>
<td>12 (54.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>8 (14%)</td>
<td>26 (46.4%)</td>
<td>22 (39.2%)</td>
</tr>
</tbody>
</table>

Among 34 HBV-DNA positive patients 26 patients (76.4%) were male and 8 patients (23.5%) were female (Table-III). Mean age of these 34 patients was 35±14 years.

Table-III: Sex distribution of HBV-DNA positive patients (n=34).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>26</td>
<td>76.5%</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>23.5%</td>
</tr>
</tbody>
</table>

Among 56 HBsAg positive patients, 15 patients were receiving anti-viral therapy. Out of them, 4 patients showed decreased viral load and HBV-DNA could be not detected in 11 patients (comparative study was done by taking the previous record of HBV-DNA load of the same patients from AFIP) (Table-IV).

Table-IV: Treatment response among the patients receiving anti-viral therapy (n=15).

<table>
<thead>
<tr>
<th>Treatment receiving</th>
<th>HBV-DNA load after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Decreased in 4 patients</td>
</tr>
<tr>
<td></td>
<td>Not detected in 11 patients</td>
</tr>
<tr>
<td>Percentage</td>
<td>26.6%</td>
</tr>
</tbody>
</table>

Discussion

The detection of viral DNA in serum is strong evidence that infectious virions are present. HBV DNA levels are detectable by 30 days following infection, generally reach a peak at the time of acute hepatitis, and gradually decrease and disappear when the infection resolves spontaneously.

In this study, among 56 HBsAg positive patients, rt PCR detected HBV-DNA in 34 (60.7%) patients and 22 (39.3%) were negative for HBV-DNA. This finding correlates with a study by Ali Koyncver which was done among HBsAg positive patients and detected HBV-DNA in the serum of 58.3% patients and 41.7% patients were negative for HBV-DNA.

Among 34 (60.7%) HBV-DNA positive patients, 8 (23.5%) were HBeAg positive which indicates that the patients may be in immune tolerance phase and 16 (47%) patients were anti-HBe positive which indicates that the patients may be in immune clearance phase. These findings correlate with the studies by Danta et al and Yun-fan et al. Again, among these 34 HBV-DNA positive patients, 26(76.5%) patients were negative for HBeAg. This correlates with the study by Maimuna E Mendy which showed that HBV-DNA was detected in 77.0% of HBeAg negative patients and all HBeAg positive patients.

On the other hand, among 22 (39.3%) HBV-DNA negative patients, none were HBeAg positive and 10 (45.5%) patients were anti-HBe positive which indicates that the patients may be of silent infection which correlates with the study of Yun-Fan et al. Twelve (54.5%) patients were negative by both the tests (HBeAg and anti-HBe) which indicates that the patients may be of precore mutant variety or carriers which correlate with the study of Yun-Fan et al.

In this study, among the 56 HBsAg positive patients, HBeAg was positive in 8(14.28%) patients which also coincides with the study by Chopra GS et al which showed that 26% patients were positive for HBeAg among HBsAg positive patients.

The present data indicates that the HBeAg and anti-HBe status do not necessarily reflect the HBV-DNA level in the serum. So, the assay may be useful for patients who are HBeAg negative but carry...
the HBV virion\textsuperscript{18}, as the precore point mutant HBVs cause HBeAg negative phenotype irrespective of their status of replication\textsuperscript{3,19}. Moreover, by Polymerase- Chain-Reaction (PCR) method, we know that 70\% to 85\% of people with anti-HBe antibodies have detectable viral DNA in the circulation to have some level of ongoing viremia\textsuperscript{20}.

Among 34 HBV-DNA positive patients, 26 (76.4\%) were male and 8 (23.5\%) were female, thus depicting male predominance. Mean age of these 34 patients was 35±14 years. These findings correlate with the study by Azita Ganji et al which also showed male predominance (72.0\%) and mean age of these subjects was 39±11 years\textsuperscript{21}. The detection and quantitation of HBV-DNA play an important role in diagnosis as well as monitoring HBV infection to assess therapeutic response to antiviral in chronic Hepatitis B patients\textsuperscript{22}. Asymptomatic HBsAg positive carriers should not be considered as having inactive disease. They should be followed up every 3 to 6 months to know the activity of the disease and development of complications. Majority of these carriers suffer from chronic hepatitis rather than acute hepatitis probably contracting the disease in perinatal period or early childhood\textsuperscript{17}.

HBV-DNA persists throughout the natural history of chronic hepatitis B, even in patients with serologic evidence of viral clearance. Treatment does not generally allow complete eradication of HBV from the organism. Continuous long-term therapy is required to maintain effective viral suppression and symptom control\textsuperscript{23}. In the present study, among the 15 treatment receiving patients, viral load was decreased in 4 (26.6\%) patients and viral load could not be detected in 11 (73.4\%) patients after effective treatment which correspond with the study by Chopra GS et al (63\%)\textsuperscript{3}. Antivirals used for these patients were nucleoside analogs such as Entecavir, Lamivudine, Telbivudine. These antivirals were used for various durations (ranging from 3 months to 10 years) according to individual patient’s requirement.

**Conclusion**

Prevalence rates of HBV vary across the world with the highest rates being observed in eastern Asia. Thus, the high sensitivity, specificity, accuracy, wide linearity, good reproducibility combined with a small sample volume requirement make this quantitative HBV rt-PCR assay in routine diagnostic laboratories well suited for application to large clinical and epidemiological studies.

**References**


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