Correlation of hepatitis B virus viral load and HBeAg status in HBsAg positive patients

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Abstract

Background: Hepatitis B virus (HBV) infection is a major cause of chronic liver disease and hepatocellular carcinoma. About one third of the world's population is infected with the HBV. To control the spread of HBV it is important to understand the molecular epidemiology of HBV. Objective: Present study was aimed to detect HBV viral load by Real Time PCR in HBsAg positive patients and to correlate with HBV markers. Material and methods: A total of 100 blood samples were obtained from HBsAg positive patients. DNA was extracted by Roche High Pure System COBAS TaqMan kit and quantified by COBAS TaqMan HBV Test Kit on COBAS TaqMan 48 analyzer (Roche). Results: Majority (48%) of the patients included in the present study were in the age group 20–40 years and most of the patients (60%) were found to be asymptomatic. Out of 100 patients, 80 (80%) were males and only 20 (20%) were females and 28 were HBeAg positive and 72 were HBeAg negative. Of above 28 HBeAg positive samples 16 (57.15%) were having viral load >20000 IU/mL and 12 (42.85%) were having <20000 IU/mL Out of 72 HBeAg negative samples 20 (27.77%) were having viral load >20000 IU/mL and 52 (72.23%) were having <20000 IU/mL. Only 6% (6/100) of the patients, the target was not in detectable (TND) range and these all were HBeAg negative. Conclusion: It's important to carry out HBV viral load testing in patients who are HBsAg positive on regular basis not only in HBeAg positive cases but in negative cases too. As on our study we found HBeAg negative patients had viral load more than 20000 IU/mL which mandates initiation of treatment.

Keywords: Hepatitis B virus, viral load, HBeAg, HBsAg, PCR
the amount of cccDNA inside hepatocytes (Brunetto, 2010; Chan et al., 2010).

Therefore, the present study was undertaken to investigate the correlation between the HBV DNA level and HBeAg status in patients attending SMS Hospital. **Material and Methods**

The present study was conducted on 100 HBsAg positive patients, whose blood samples were received for HBV DNA viral load in the Advance Research Laboratory in the Department of Microbiology and Immunology, SMS Medical College, Jaipur from different Wards and OPDs of SMS hospital and allied hospitals.

**Material and Methods**

**Blood (plasma/serum):** A total 5 mL of blood was collected in an EDTA (ethylenediaminetetraacetic acid) vial for PCR and in plain vial for serology. The blood was centrifuged and plasma/serum was transferred to a sterile eppendorf tube for storage. Samples were stored in freezer at -20°C till they were tested.

**HBeAg detection:** Antigen was detected by enzyme-linked Immunosorbent assay (ELISA) using Dia.Pro ELISA kits as per manufacturer’s instructions.

**Extraction and Quantification of HBV DNA**

DNA was extracted by Roche High Pure System COBAS TaqMan kit and quantified by COBAS TaqMan HBV Test Kit on COBAS TaqMan 48 analyzer (Roche) as per manufacturer’s instructions. The HBV DNA titre is expressed in International Units (IU)/mL.

Initial activation of UNG (Uracil-N-glycosylase) was done at 50°C for 12 min followed by Activation of Taq Polymerase & inactivation of UNG at 95°C for 10 min. Cycling profile for PCR was 30 cycles of denaturation at 95°C for 16 s and annealing at 60°C for 1 min.

**Results and discussion**

Majority (48%) of the patients included in the present study were adults and most of the patients (60%) were found to be asymptomatic. Out of 100 patients, 80 (80%) were males and only 20 (20%) were females. Out of 100 samples 28 were HBeAg positive and 72 were HBeAg negative. Out of 100 patients, in 86% (86/100) the mode of transmission was unknown and majority of patients belonged to the asymptomatic group. The mode of transmission by HRSC, blood transfusion and perinatal route were 2%, 10% and 2% respectively (Table 1).

Of above mentioned 28 HBeAg positive samples 16 (57.15%) were having viral load >20000 IU/mL and 12 (42.85%) were having viral load <20000 IU/mL. Out of 72 HBeAg negative samples 20 (27.77%) were having viral load >20000 IU/mL and 52 (72.23%) were having viral load <20000 IU/mL. In 26% (26/100) of the patients, the target was not in detectable/ very low (below 29 IU/ML) range and these all were HBeAg negative (Table 2).

In present study we studied correlation of serum HBeAg and HBV DNA levels as both are used biomarkers to monitor the disease course and response to treatment in HBV positive patients.

In present study, 82% of patients were adults. Similarly various studies have reported that adults men or older were most commonly affected with HBV (Attaullah et al., 2011; Karki et al., 2008). The prevalence of HBV has been reported to be significantly elevated in males then females, similar findings have been reported from India (Chattopadhyay et al., 2006; Perumal et al., 2004; Attaullah et al., 2011). This could be due to higher prevalence of infection in adult males as they are more exposed to the risk factors.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total patients</th>
<th>Asymptomatic</th>
<th>Symptomatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>18</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Adults</td>
<td>82</td>
<td>50</td>
<td>32</td>
</tr>
<tr>
<td>Male</td>
<td>80</td>
<td>52</td>
<td>28</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>HRSC (high risk sexual contact)</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>BT (blood transfusion)</td>
<td>10</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Unknown (incidentally detected)</td>
<td>86</td>
<td>38</td>
<td>28</td>
</tr>
<tr>
<td>Perinatal</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1. Age wise, sex wise and mode of transmission wise distribution of patients
In present study we observed that the HBV DNA load was high in HBeAg positive cases, and good correlation was seen between the two, but in 26% of HBeAg negative cases the HBV DNA levels were > 20,000 IU/mL which is the level at which treatment is started, monitoring these patients only on basis of HBeAg can be misleading in such cases. Such findings are generally observed in patients with precore mutations (Ghabeshi et al., 2013). Moreover 46 (62.16%) HBeAg negative patients had HBV DNA level more than 29 IU/mL. Therefore only HBeAg cannot be used as marker for monitoring CHB patients. However as in these patients HBeAg status was done only once, which can be misleading, it is recommended to do HBeAg testing at least twice to predict response of CHB patients. Another limitation of the study was that sequencing was not done to know the presence of precore mutations.

Certain studies have even used HBsAg quantitation by chemiluminescence, Architect QT (Abbott Laboratories) and Elecsys HBsAg II quant (Roche Diagnostics) to monitor response to treatment and correlate level with HBV DNA though some authors have reported contradicting results also, whether HBsAg and HBeAg detection correlates with HBV DNA (Kuhns et al., 2004; Wiegand et al., 2008), but most studies have shown significant correlation between these values (Manesis et al., 2007; Moucari et al., 2009; Brunetto et al., 2009).

Several studies show that HBsAg and HBV DNA levels change during different phases of CHB (Nguyen et al., 2010; Jaroszewicz et al., 2010; Chan et al., 2011). Without treatment, the serum HBsAg level changes slower than the HBV DNA level (Chan et al., 2010; Nguyen et al., 2010). Recently HBsAg level has been used for differentiating between inactive carriers and patients with active disease. Most inactive carriers of HBV infection have a low HBsAg level (Chan et al., 2010).

There have been many limitations in our study, we had not done HBsAg quantitation by chemiluminescence which would have helped understand correlation of HBsAg HBeAg and HBV DNA levels, moreover sequencing of precore mutations would also help us understand the correlation better.

**Conclusion**

It’s important to carry out HBV viral load testing in patients who are HBsAg positive on regular basis not only in HBeAg positive cases but in negative cases too. As in our study, we found that 26% HBeAg negative patients had viral load more than 20000 IU/mL which mandates initiation of treatment and only 26% patients had viral load below 6 IU/mL or TND in HBeAg cases.

**Acknowledgment**

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**Conflicts of interest**

The author declares no conflict of interest.

**References**


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