Liver Histopathological Features of HBeAg-Negative Chronic Hepatitis B in Young Bangladeshis

Mamun-Al-Mahtab 1*, Salimur Rahman 2, Mobin Khan 1, Fazal Karim 2, Md. Kamal 3

1 Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
2 Dhaka Mahnagar Hospital, Dhaka, Bangladesh
3 Department of Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

Background and Aims: Hepatitis B virus (HBV) infection is common in Bangladesh. However, the characteristics of young patients incidentally detected with hepatitis B e antigen (HBeAg)-negative chronic hepatitis B is yet to be studied in this country.

Methods: We did percutaneous liver biopsies in 36 patients with chronic hepatitis B (CHB) aged between 8 and 20 years. They were all HBeAg-negative with persistently normal or raised serum alanine aminotransferase (ALT) values.

Results: 56% of patients had significant necro-inflammation. Significant fibrosis was seen in 17.6% of patients. Serum ALT was raised in 38.2%. A high HBV DNA load was observed only in 26.5% of patients. HBeAg-negative CHB is assumed not to be present in the age group studied, since this is the age group that represents immunotolerance to CHB. Immunotolerant CHB patients are positive for HBeAg, have normal ALT with high HBV DNA load. However, our study not only documented that HBeAg-negative CHB is common in our young population, but the data were also in contrary to what was expected in such age group of CHB patients.

Conclusions: HBeAg-negative CHB is an entity that can be seen in young population and a significant percentage of such patients may have considerable hepatic involvement.

Keywords: Chronic Hepatitis B, HBeAg Negative, Liver Histology
like Bangladesh, lifetime risk of acquiring HBV infection is above 40% and transmission of HBV is both horizontal and vertical.

The precore/core region of the HBV genome encodes the nucleocapsid protein (HBcAg) and hepatitis B e antigen (HBeAg) (3, 4). The core open reading frame has two transcripts with heterogeneous 5' ends and two in-phase initiation codons. HBeAg is translated from the precore mRNA producing a precursor polypeptide comprising the precore and the entire core region. The precore polypeptide is translocated into the endoplasmic reticulum by a signal peptide. Cleavage of the amino and carboxy termini results in a secretory protein HBeAg. HBcAg is translated from the pregenomic RNA.

The biological role of HBeAg in the HBV replication cycle is uncertain. Expression of HBeAg is non-essential for virus replication in animal models (5) and in humans (6). It has been suggested that HBeAg may act as a tolerogen or a target for immune response. In utero exposure to HBeAg can induce immune tolerance in newborn mice (7). Perinatal transmission of HBV from HBeAg-positive mothers results in chronic HBV infection in the majority of babies (8). In addition, HBeAg appears to modulate the host's immune response (8-15). Precore variants that do not produce HBeAg may be selected because they can evade the immune clearance.

Mutations in the precore region of the HBV genome have been described (13-15). It results in HBeAg-negative HBV infection. The predominant mutation involves a G to A change at nucleotide 1896 (G1896A). This results in a premature stop codon (eW28X) and prevents translation of the precore protein, thus, the production of HBeAg is completely abolished (16).

The core promoter region (nucleotides 1742 to 1849) is located upstream of the precore region (nucleotides 1814 to 1901). It has an important role in HBV replication as well as HBeAg production (17). Mutations in these regions down-regulate the precore mRNA transcription and HBeAg synthesis (18, 19). The most common core promoter variant results from a substitution in A to T at nucleotide 1762, and G to A at nucleotide 1764 (A1762T, G1764A) (20-22). These changes commonly lead to HBeAg-negative HBV infection (20).

HBeAg-negative chronic hepatitis B (CHB) in the young is deemed to be unusual given the fact that they tend to be immunotolerant with normal serum alanine aminotransferase (ALT), high HBV DNA load and HBeAg-positivity. However, since HBeAg-negative CHB is not infrequently observed in our clinical practice, we conducted this study to assess the extent of hepatic histopathologic involvement in this group of patients.

**Patients and Methods**

Patients with chronic HBV infection (HBsAg positive for at least six months) attending our OPD clinics in Dhaka, Bangladesh were studied prospectively. They were between 8 and 20 years of age. They were all treatment naïve patients with no past history of receiving any anti-viral medications. Written informed consent was obtained from each patient. In case of minors, the consent was obtained from their legal guardians.

The patients had to be negative for antibodies to hepatitis C virus (anti-HCV) and positive for serum HBV DNA by polymerase chain reaction (PCR) assay; they were HBeAg-negative and enrolled irrespective of their liver enzyme levels. Patients with clinical evidence of liver cirrhosis were excluded.

In all of participants, serum ALT level and platelet count were measured using an autoanalyzer and prothrombin time by Quick’s method. The cut-off value for abnormal ALT was set at 42 U/L. HBeAg was checked by enzyme-linked immunosorbent assay (ELISA) (Abbott Labs, Chicago). Anti-HCV was measured by third generation ELISA (Abbott Labs, Chicago). Co-infection with hepatitis D virus, however, could not be excluded, as this test was not available in Bangladesh. HBV DNA quantification was done by PCR (Amplicon HBV Monitor Assay, reverse transcriptase (RT)-PCR, Roche Molecular Systems, California). The lower limit of detection was 250 copies/mL. HBV DNA >10^5 copies/mL was considered as a "high DNA load.”

All patients underwent percutaneous liver biopsies done using a Tru-Cut biopsy needle under local anaesthesia. Any patient with detectable HBV DNA by PCR was selected to undergo liver biopsy irrespective of their HBV DNA load; anti-HBe status and/or serum ALT level were also measured for these patients. We checked the prothrombin time and platelet count in every patient within a week before the procedure. Liver biopsies were done if the baseline prothrombin time was not prolonged more than three sec than the control value and the platelet count was not less than 100,000/mm3. The patients were followed at 15-min intervals for one hr and then at 30-min intervals for another two hrs. Patients were discharged 24 hrs post-liver biopsy. Biopsies were scored using histologic activity index (HAI) score by a single histopathologist who was unaware of the clinical and laboratory data of the patients.
Results

A total of 34 patients (12 women and 22 men) were studied. They were between 8 and 20 years of age. The patients’ characteristics are shown in Table 1. After liver biopsy, no complications but occasional complaints of mild right upper abdominal or right shoulder tip pain in few cases, was reported. Results showed that significant hepatic necro-inflammation (i.e., HAI-N1>3) was seen in 19 (56%) of 34 patients, while significant fibrosis (i.e., HAI-F ≥3) was seen in six (18%).

Table 1. Baseline characteristics of the study population.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>34</td>
</tr>
<tr>
<td>Age range (yrs)</td>
<td>8-20</td>
</tr>
<tr>
<td>Gender (male:female)</td>
<td>22:12</td>
</tr>
<tr>
<td>Serum ALT</td>
<td>17 to 95 U/L</td>
</tr>
<tr>
<td>HBV DNA load</td>
<td>10^3 to 10^7 copies/mL</td>
</tr>
<tr>
<td>HAI-N1 score range</td>
<td>1-9</td>
</tr>
<tr>
<td>HAI-F score range</td>
<td>0-3</td>
</tr>
</tbody>
</table>

Thirteen (38%) of 34 had elevated serum ALT. High HBV DNA load (>10^5 copies/mL) was seen in nine (26%) patients.

Discussion

HBeAg-negative CHB is assumed not to be present in the age group studied, since this is the age group that represents immunotolerance to CHB. Immunotolerant CHB patients are positive for HBeAg, have normal ALT with high HBV DNA load. However, our study not only documented that HBeAg-negative CHB is common in our young population, but the data were also in contrary to what was expected in such age group of CHB patients. We observed significant hepatic involvement, especially necro-inflammation type and significant fibrosis in the majority of our patients. Most of our patients had low HBV DNA load, while a remarkable percentage had elevated ALT.

Scarce information is available in the literature about HBeAg-negative CHB in young population or the extent of histologic involvement of liver in them. A Korean group in 2004 studied 155 young men with chronic HBV infection in whom 102 were found HBeAg-positive. They also demonstrated a lower fibrosis score in those with HBeAg-positive chronic HBV infection than patients with HBeAg-negative CHB (23).

There are however, several reports comparing hepatic histology between HBeAg-positive and -negative CHB patients. It has been observed in studies from different countries that HBeAg-negative CHB tends to cause more severe liver disease. A larger Turkish study in 2003, included 354 patients with CHB and revealed a significantly more necro-inflammation and fibrosis in HBeAg-negative CHB than in HBeAg-positive patients (24). The Egyptians report similar observation of less severe histologic liver disease in HBeAg-positive CHB compared to HBeAg-negative patients in their series of 670 patients (25). The result of the observations by the Greeks is also not different from ours (26).

The Indian experience with HBeAg-negative CHB is also not different from other observers in various countries. In 2004, a group from G.B. Pant Hospital, New Delhi, studied 60 patients with CHB and demonstrated a statistically significant difference in liver fibrosis between HBeAg-positive and -negative CHB patients with fibrosis score being higher in the HBeAg-negative group. These patients also had higher degrees of hepatic necro-inflammation than their HBeAg-positive counterparts (27).

In one of our studies where we recruited 80 CHB patients, we found that 8% patients with HBeAg-positive CHB had minimal chronic hepatitis, 69% had mild chronic hepatitis, 19% had moderate chronic hepatitis, while severe chronic hepatitis was seen in 4%. In case of HBeAg-negative CHB, these figures were 11%, 54%, 25%, and 11%, respectively (28). Later, we studied a larger sample size and compared not only the degree of hepatic necro-inflammation, but also fibrosis between HBeAg-positive and -negative CHB patients. This study included 155 patients-102 HBeAg-positive and 55 HBeAg-negative patients. We found that 20.8% of patients with HBeAg-negative CHB had moderate to severe chronic hepatitis (CH). In contrast, moderate to severe CH was seen in 18.6% of patients with HBeAg-positive CHB. Significant hepatic fibrosis (i.e., HAI-F score ≥3) was also more frequent in the HBeAg-negative CHB group. In this group, 28.3% of patients had significant hepatic fibrosis as opposed to 19.6% of patients with HBeAg-positive CHB. In both of these studies, we observed that patients with HBeAg-negative CHB tend to develop more severe hepatic histologic involvement than their HBeAg-positive counterparts (29).

In one of our more recent works, we studied 42 HBeAg-negative CHB patients with very low HBV DNA load (<10^5 copies/mL) and found that even in...
them, 26% of patients had significant hepatic necroinflammation (i.e., HAI-NI score 4-8) while significant fibrosis was seen in 19% of patients (30).

The ultimate goal of therapy in CHB is to prevent morbidity and mortality related to the development of cirrhosis and HCC. In young patients with CHB, durable response rates associated with interferon-alpha (IFN-α) therapy seem to be higher than those associated with lamivudine therapy, although IFN-α is associated with higher toxicity. Clearance of HBeAg has been reported in one-third of IFN-α treated children, compared with 10% of untreated control subjects (31). There are few clinical outcome data on children that include histologic analysis in those treated with IFN (32). These data show significant difference in inflammatory response in the whole group, especially the responders 12 months post-treatment.

The use of lamivudine has shown to reduce the likelihood of development of HCC in adults (33). However, children are unlikely to continue long-term therapy because of the potential for the development of resistance. In addition, the effect of long-term antiviral therapy, particularly if started in childhood, is not known.

An interesting paper (34) on vertically-infected children who were HBeAg-positive with normal ALT and high DNA viral loads revealed 17% HBeAg clearance when treated with both lamivudine and IFN-α. All four in whom the HBeAg was cleared were infected with genotype B Asian (China and Southeast Asia) viruses and female. This paper challenges the view that perinatally-infected immunotolerant children are unlikely to respond to antiviral therapy.

It is extremely important to select the right candidates for treatment. HBeAg-negative CHB should be investigated thoroughly and managed appropriately or else many patients may progress to developing more serious liver disease. A recent paper from Taiwan reports that the cumulative probability of hepatitis relapse in HBeAg carriers is 26.9% in men and 12.5% in women over a 20-year follow-up. Moreover, 1.14% of patients included in the study progressed to cirrhosis per annum. The sample size in this study was 1241 (35).

Conclusions

Our study showed that apparently-healthy young CHB population with favorable clinical and laboratory parameters may have significant hepatic histologic involvement. There is a need for an effective strategy to achieve seroconversion in children to improve long-term outcomes without “closing off” therapeutic options. Such a strategy is likely to evolve in the next few years as the results of combination therapies including interferon and anti-viral drugs are being evaluated.

References

17. Yuh CH, Chang YL, Ting LP. Transcriptional regulation of


