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Study of hepatic histomorphology in HBeAg+ and HBeAg- patients with CHB: Experience from Bangladesh

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ABSTRACT

Objective: To compare Knodell and HAI scores in patients with wild type and pre-core/core promoter mutant CHB to see if there is any difference in severity of liver injury between these two types of HBV. **Methods:** We did percutaneous liver biopsies of 155 CHB patients. Of them 102 (65.8%) were infected wild type HBV and the rest 53 (34.2%) were infected with pre-core/core promoter mutant CHB. **Results:** 11/53 (20.8%) patients with pre-core/core promoter mutant CHB had moderate to severe CH (HAI score 8-18). In contrast, moderate to severe CH was seen in 19/102 (18.6%) patients with wild type CHB. Fibrosis score was >2 in 15/53 (28.3%) pre-core/core promoter mutant CHB as opposed to 20/102 (19.6%) patients with wild type CHB. **Conclusions:** The study shows that pre-core/core promoter mutant HBV produces more severe histological liver disease compared to wild type HBV.

1. Introduction

Hepatitis B virus (HBV) is a double-stranded DNA virus that belongs to the family of hepadnaviruses. Other members of the family include Peaking duck hepatitis virus, woodchuck hepatitis virus and ground squirrel hepatitis virus[1]. Blumberg and colleagues identified HBsAg in Philadelphia in 1965 in the blood of an Australian aborigine, the reason why HBsAg was initially named Australia antigen[2]. Blumberg received Noble Prize in Medicine in 1977 for this ground breaking discovery.

HBV infects nearly 350 million people worldwide. The clinical manifestations vary widely with asymptomatic acute viral B hepatitis on one end and hepatocellular carcinoma (HCC) on the other end of the spectrum. There are about 400 million HBV careers worldwide. Of them 75%-80% reside in Asia and Western Pacific. HBV is responsible for over 1 million deaths per year globally. It is a major cause of cirrhosis of liver and HCC worldwide[1].

HBV is mainly transmitted by percutaneous and membrane exposure to infected body fluids. HBsAg and HBV DNA by PCR have been identified in most body secretions e.g. blood, saliva, menstrual and vaginal discharges, seminal fluid and serous discharges with the exception of stool[1]. HBV replicates in hepatocytes, but HBV encoded proteins have been identified in other body tissues like testes, stomach, colon, kidney, bone marrow, peripheral mononuclear cells, nerve ganglia and skin, which represent large extra-hepatic reservoir of HBV[3].

HBV is transmitted from infected mother to neonate

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in or around the time of birth. 60%-90% babies born to HBeAg positive mothers and 15%-20% born to HBeAg negative mothers become infected respectively. There is also risk of transmission of HBV if the pregnant mother has acute viral B hepatitis in second or third trimester or within two months of labour. HBV has been detected in breast milk, but it is probably not transmitted through breast milk[3].

HBV is one of the commonest causes of post-transfusion hepatitis. Intravenous drug abuse is also an important mode of transmission of HBV. A single needle stick injury from a patient positive for HBeAg is associated with 30% risk of acquiring the infection. Sexual transmission of HBV is another important route of spread of the virus[1]. Dentists and barbers are likely sources of some HBV infections. Tattooing, acupuncture, body piercing, artificial insemination and organ transplantation etc. are also less frequent sources of HBV infection[3].

In intermediate prevalence countries like Bangladesh, lifetime risk of acquiring HBV infection is above 40% and transmission of HBV is both vertical and horizontal. In Europe and in North America, sexual route is the main route of transmission of HBV. It occurs in individuals predominantly below 50 years of age. Needle sharing among intra-venous drug abusers and occupational exposure to contaminated blood and blood products also remain important routes of spread for the virus[3].

The precore/core region of the HBV genome encodes the nucleocapsid protein (HBcAg) and HBeAg[4,5]. 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 nonessential for virus replication in animal models[6] and in humans[7]. 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[8]. Perinatal transmission of HBV from HBeAg-positive mothers results in chronic HBV infection in the majority of babies[9]. In addition, HBeAg appears to modulate the host's immune response[10-13]. Precore variants that do not produce HBeAg may be selected because they can evade immune clearance.

Mutations in the precore region of the HBV genome have been described[14-16]. It results in HBeAg negative HBV infection. The predominant mutation involves a G to A change at nucleotide 1 896 (G1896A). This results in a premature stop codon (eW28X) and prevents translation of the precore protein. Thus the production of HBeAg is completely abolished[17].

Core promoter region (nucleotides 1 742 to 1 849) is located upstream of the precore region (nucleotides 1 814 to 1 901). It has important role in HBV replication as well as HBeAg production[18]. Mutations in these regions downregulate precore mRNA transcription and HBeAg synthesis[19,20]. The most common core promoter variant results in substitution in A to T at nucleotide 1 762 and G to A at nucleotide 1 764 (A1762T, G1764A)[21-23]. These changes commonly leads to HBeAg negative HBV infection[21].

2. Material and methods

Patients with chronic HBV infection (HBsAg positive for at least 6 months) attending our OPDs in Dhaka, Bangladesh between April 2010 and April 2012 were studied prospectively. Written informed consent was obtained from each patient. The patients had to be negative for anti-HCV antibody and positive for serum HBV DNA (>1×10^5 copies/mL) using a DNA hybridization assay (Digene Hydrid Capture2 system; Digene Corporation, Gaithersburg, Maryland, USA); they were enrolled irrespective of their HBeAg status and liver enzyme levels. Patients with clinical evidence of liver cirrhosis were excluded.

All patients underwent percutaneous liver biopsy. Biopsies were done using trucut biopsy needle under local anaesthesia. Patient characteristics are shown in Table 1. We checked prothrombin time, platelet count, bleeding time and clotting time in every patient within a week before the procedure. Liver biopsies were done if baseline prothrombin time was not prolonged more than 3 s beyond control value, platelet count was not less than 100 000/mm^3 and bleeding and clotting times were within normal limits. None of the patients experienced any complication except for the occasional complaint of mild right upper abdominal or right shoulder tip pain in few cases. The patients were followed up at 15 min intervals for 1 h and then at 30 min intervals for another 2 h. Bed rest was given for 24 h and patients were

discharged 48 hours post-liver biopsy. Biopsies were scored using Knodell score and histologic activity index (HAI) score.

Table 1
Characteristics of study population.

Parameter	Value
Total patients	155
Age (years)	25 (8-55)
Gender (Male: Female)	119:36
Wild type	102
Precore/Core promoter mutant	53
HAI score	1-18
Fibrosis score	0-4

3. Results

A total of 155 patients were studied. Results in HBeAg positive wild type CHB show that HAI score (i.e. necro-inflammatory score) was between 1-3 in 39/102 patients, between 4-8 in 44/102 patients, between 9-12 in 18/102 patients and 1/102 patients had score between 13-18. In HBeAg negative pre-core/core promoter mutant CHB, these figures are 26/53, 16/53, 8/53 and 3/53, respectively.

38.2% wild type CHB patients included in this study had minimal chronic hepatitis, 43.1% had mild chronic hepatitis and 17.6% had moderate chronic hepatitis while severe chronic hepatitis was present in 0.9% patients. In pre-core/core promoter mutant CHB patients, these figures were 49%, 30.2%, 15.1% and 5.6%, respectively.

Fibrosis score was <2 and >2 in 82/102 and 20/102 patients with wild type CHB and in pre-core/core promoter mutant CHB, it was <2 and >2 in 38/53 and 15/53 patients respectively.

80.4% patients with wild type CHB had fibrosis score <2 and 19.6% had >2. In case of pre-core/core promoter mutant CHB, it was 71.7% and 28.3% respectively.

4. Discussion

Our study reveals that CHB patients infected with core/core promoter mutant HBV tend to develop more severe necro-inflammation and fibrosis in the liver compared to those who are infected with wild type HBV.

In 2004, an Indian study involving 60 patients conducted by a group from G.B. Pant Hospital, New Delhi demonstrated statistically significant difference in liver fibrosis between wild type and core/core promoter mutant CBH patients, with fibrosis score being higher in core/core promoter mutant CHB. Although these patients also had higher HAI score than those infected with wild type HBV, the difference was not statistically significant[24].

A Korean study in 2004 also yielded similar results. The study included chronic HBV infected 85 young, male patients and demonstrated lower fibrosis score in those wild type HBV infection than those with core/core promoter mutant HBV infection. Here also the correlation was not significant statistically in case of HAI score[25].

A Turkish study in 2003, that included 354 CHB patients revealed significant difference between necro-inflammatory activity and precore/core promoter mutant and wild type CHB. However the difference in fibrosis was not significant[26].

In one of our previous works where we recruited 80 CHB patients, it was seen that 7.69% patients with wild type CHB had minimal chronic hepatitis, 69.23% had mild chronic hepatitis, 19.23% had moderate chronic hepatitis 3.85% had severe chronic hepatitis. In case of pre-core/core promoter mutant CHB these figures were 10.71%, 53.57%, 25% and 10.71%, respectively. Our study showed that patients with pre-core/core promoter mutant CHB tend to develop moderate to severe chronic hepatitis more[27].

In conclusion our study shows that core/core promoter mutant HBV infection leads to more marked necro-inflammation and fibrosis in the liver compared wild type HBV infection. Out finding is more or less consistent with similar studies carried our elsewhere as well as in Bangladesh. This may well explain disease progression and lack of response to anti-viral treatment in HBeAg negative CHB patients.

Conflict of interest statement

We declare that we have no conflict of interest.

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