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OBSERVATIONAL STUDY

Early termination of immune tolerance state of hepatitis B virus infection explains liver damage

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Abstract

AIM: To assess an early termination of immune tolerance state of chronic hepatitis B virus infection in Bangladesh and its clinical significance.

METHODS: From a series of 167 treatment-naive chronic hepatitis B patients aged between 12 to 20 years (mean ± SD; 17.5 ± 2.8 years), percutaneous liver biopsies of 89 patients who were all hepatitis B e antigen negative at presentation were done. Of them, 81 were included in the study. They had persistently normal or raised serum alanine aminotransferase (ALT) values. A precore mutation (PCM) study was accomplished in 8 patients who were randomly selected.

RESULTS: Forty-four (53.7%) patients had significant necroinflammation (HAI-NI > 7), while significant fibrosis (HAI-F ≥ 3) was seen in 15 (18.5%) patients. Serum ALT (cut off 42 U/L) was raised in 29 (35.8%) patients, while low HBV DNA load (< 10^5 copies/mL) was observed in 57 (70.4%) patients. PCM was negative in all 8 patients.

CONCLUSION: This study indicates that the current concept of age-related immune tolerance state of HBV infection deserves further analyses in different population groups.

Key words: Chronic hepatitis B; Immune tolerance; Early termination

Core tip: Immune tolerance phase usually prevails for up to 20-25 years in subjects with chronic hepatitis B virus (HBV) infection. However, the present study showed that considerable numbers of chronic HBV-infected patients of Bangladesh lost hepatitis B e antigen and developed anti-HBe. Early termination of immune tolerance phase of these young patients was also associated with elevated alanine aminotransferase, hepatic necroinflammation and considerable hepatic fibrosis in some patients. Treatment guidelines are warranted for these patients as there is a paucity of information about their entity.

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INTRODUCTION

Maybe nearly 2 billion people have been infected with hepatitis B virus (HBV) worldwide. The clinical manifestations vary widely with asymptomatic acute viral B hepatitis at one end and hepatocellular carcinoma (HCC) at

the other. There are about 400 million chronic HBV carriers worldwide. Of them, 75%-80% resides in Asia and the Western Pacific region. HBV is responsible for over one million deaths per year globally. It is a major cause of cirrhosis of liver and HCC worldwide[1].

Although there is a paucity of information about a nation-wide survey regarding HBV prevalence in Bangladesh, published data show that about 5%-6% of apparently healthy individuals are HBV carriers in Bangladesh[2-4]. There may be about 6-8 million chronic HBV carriers in Bangladesh and most of them are unaware of their disease. In intermediate prevalence countries like Bangladesh, lifetime risk of acquiring HBV infection is above 40%[1].

The precore/core region of the HBV genome encodes the nucleocapsid protein (HBcAg) and hepatitis B e antigen (HBeAg)[5,6]. The biological role of HBeAg in HBV replication cycle is uncertain. Expression of HBeAg is nonessential for virus replication in animal models[7] and in humans[8]. In utero exposure to HBeAg can induce immune tolerance in newborn mice[9]. Mutations in the precore region of the HBV genome have been described[10-12] resulting in HBeAg negative HBV infection. The core promoter region (nucleotides 1742 to 1849) has an important role in HBV replication as well as HBeAg production[13] and mutations in this region commonly lead to HBeAg negative HBV infection[14].

Chronic HBV infection can be divided into different phases, which may not be sequential. Patients may present: (1) in a state of immune tolerance; (2) with hepatitis B e antigen (HBeAg)-positive chronic HBV; (3) with HBeAg-negative chronic HBV; or (4) as an inactive hepatitis B surface antigen (HBsAg) carrier. A state of immune tolerance with minimum liver damage is usually seen in chronic HBV carriers until 25 years of age.

The present study was accomplished to evaluate the biochemical, virological and immunological statuses of young chronic HBV carriers in Bangladesh. It seems that there may be an early termination of immune tolerance state of HBV in Bangladesh. However, there is no therapeutic recommendation for these young HBV-infected patients. Here, we provide evidence suggesting that considerable numbers of these patients should be treated as otherwise they may develop complications of chronic HBV infection.

MATERIALS AND METHODS

Patients

One hundred and sixty-seven treatment naive young chronic HBV-infected patients, aged between 12 and 20 years (17.5 ± 2.8 years, n = 167), were enrolled in this study. At presentation they were asymptomatic with no features of chronic liver disease. They were all HBsAg positive either at vaccination, school health screening or family screening. All of them had at least two HBsAg positive results at a minimum of 6 mo apart.

Of these patients, 89 were HBeAg negative, while the others tested positive for HBeAg. They were all negative for serological markers of hepatitis C virus, IgM antibodies to hepatitis A virus and hepatitis E viruses. Also, they had no history of alcohol consumption and no evidence of pregnancy. None of the patients had received an antiviral drug for treatment of HBV infection. The Ethical Committee of Farabi General Hospital, Dhaka, Bangladesh gave ethical approval for the study. The nature and purpose of the study were explained to all patients or their guardians in the case of minors. Informed written consent for undergoing percutaneous liver biopsy was obtained. Patients were excluded from further analyses if an adequate amount of liver tissue (*i.e.*, 1.0 cm) was not available at liver biopsy[15]. Eight patients were excluded from final analyses as adequate amounts of liver tissue were not available from them. Thus, a total of 81 HBeAg negative chronic hepatitis B (CHB) patients were included for final analyses.

Biochemical and serological tests

The level of ALT in serum was measured by auto-analyzer (Microlab 300, Vitalab Micro Series, Vital Scientific NV, The Netherlands). The cut off for the upper limit of normal (ULN) was ALT 42 U/L. HBsAg was assessed using an ELISA kit (Diasorin, Fallugia, Italy). HBeAg was checked in the sera using an ELISA kit (Abbott Labs, Chicago, IL, USA).

Quantification of serum HBV DNA level

First, HBV DNA was extracted from the patient's serum. It was then amplified by polymerase chain reaction (PCR) and detected using fluorescent reporter dye probes specific for HBV (Amplicon HBV Monitor Assay, Roche Molecular Systems, CA, United States). The lower limit of detection was 250 copies of HBV DNA/mL.

Amplification of the pre-core region by the PCR

Oligonucleotide primers were synthesized in a 380B DNA synthesizer (Applied Biosystems Japan, Tokyo, Japan). PCR was performed by a modification of the procedure originally described by Saiki *et al*[16]. Briefly, 10 uL of the DNA sample was heated at 95 ℃ for 7 min to denature proteases, spun in a microcentrifuge for 5 seconds and allowed to cool at room temperature. Target sequences were amplified in a 100-pd reaction volume with the use of the Gene Amp DNA amplification reagent kit (Perkin-Elmer Cetus, Norwalk, Conn., United States), as recommended by the manufacturer. The amplification was carried on for 30 cycles in a programmable DNA thermal cycler (Perkin-Elmer Cetus). The reaction was allowed to proceed at 94 ℃ for 1 min, at 55 ℃ for 1.5 min, and at 72 ℃ for 3 min in each cycle. In the last cycle, the reaction at 72 ℃ was continued for 10 min to ensure complete DNA extension.

Liver biopsy

Percutaneous liver biopsy was performed under local anesthesia using a 16G Tru-cut biopsy needle (Cardinal Health, McGaw Park, IL, United States). A biopsy specimen of more than 1.0 cm in length with five to six portal

Table 1 Baseline characteristics of study population

Parameters	
Total number of patients	81
Male	60[a] (74%)
Female	21 (26%)
Age (yr)	17.5 ± 2.8 (12-20)
ALT ⩽ 42 (U/L)	52[a] (65.2%) (13-42)
ALT > 42 (U/L)	29 (34.8%) (44-281)
DNA ⩽ 100000 (copies/mL)	57[a] (70.4%)
DNA > 100000 (copies/mL)	24 (29.6%)
Non-significant hepatic necroinflammation (HAI-NI ⩽ 7)	44 (53.8%)
Significant hepatic necroinflammation (HAI-NI > 7)	37 (45.7%)
Non-significant hepatic fibrosis (HAI-F < 3)	66[a] (81%)
Significant hepatic fibrosis (HAI-F ⩾ 3)	15 (18.5%)
Cirrhosis	2/15

Figure in the round bracket indicates the percentage and the square bracket indicates range. [a]$P < 0.05$ *vs* same parameter. HAI-NI: Histologic activity index-necroinflamation; ALT: Alaninaminotransferase; HAI-F: Histologic activity index-fibrosis.

tracts was evaluated. Histology was graded according to histological activity index (HAI) using the criteria of Knodell *et al*[17]. The total HAI score comprises necroinflammation (HAI-NI) and fibrosis (HAI-F) scores. The HAI-NI scale includes three components (0-10, piecemeal necrosis; 0-4, lobular necrosis and inflammation; 0-4, portal inflammation). HAI-F was graded according to severity: 0, absence of fibrosis; 1, fibrous portal expansion; 3, bridging fibrosis; 4, cirrhosis.

Statistical analysis

Data are shown as mean ± SD. Means were compared using the Student's *t* test. For differences determined by the *F* test, the t test was adjusted for unequal variances (Mann-Whitney's *U* test). $P < 0.05$ was considered statistically significant.

RESULTS

A total of 81 patients with HBeAg-negative chronic HBV infection were enrolled in this study as a sufficient amount of liver biopsy specimens could be collected from these patients. The baseline data of these patients is given in Table 1. Sixty of them (74%) were male and the remaining 21 were female (26%). The numbers of male patients were significantly higher than females (60 *vs* 21, $P < 0.05$). The age of the patients was 17.5 ± 2.8 years ($n = 81$). The levels of ALT were below ULN in 52 patients (65.2%) (28.7 ± 8.6 IU/L, range, 13-42 IU/L) and ALT levels were above ULN in the remaining 29 patients (34.8%) (79.7 ± 47.4 IU/L; range, 44-281 IU/L, $P < 0.05$). The levels of HBV DNA varied considerably among patients, ranging from 779 copies/mL to 1.4×10^{12} copies/mL. In 57 patients (70.4%), the levels of HBV DNA were less than 100000 copies/mL whereas these were more than 100000 copies/mL in 24 patients (29.6%). Considering 100000 copies HBV DNA as a cut-off point of "high level" of HBV DNA, significantly higher levels of patients had low levels of HBV DNA (HBV DNA < 100000 copies/mL) compared to patients with high levels of HBV DNA (HBV DNA > 100000 copies/mL) ($P < 0.05$).

Significant levels of hepatic necroinflammation (HAI-N1 > 7) were detected in 37 of 81 patients (46%) (Table 1) and this was not statistically different with patients with low hepatic necroinflammation (44 patients, $P > 0.05$). Significant levels of hepatic fibrosis (HAI-F ⩾ 3) were detected in the liver biopsy specimens of 15 patients (19%). Among these, cirrhosis of liver was detected in two patients (Table 1).

DISCUSSION

Our study reveals that young HBeAg negative CHB patients can have significant necroinflammation and/or fibrosis in the liver. This is in contrast to our existing understanding of clinical course of chronic HBV infection that patients in the immunotolerance age group tend to have no significant hepatic pathology.

Although the study shows that a significant proportion of our patients were at risk of developing more severe liver diseases, they were not aware of this scenario. More importantly, no major guideline recommends treatment of this group of patients[18]. Similar studies have been conducted in different parts of the world to assess the extent of a similar scenario. Kumar *et al*[19] from India showed that more than 50% of their 1387 incidentally-detected chronic HBV carriers had evidence of progressive liver diseases for which treatment was indicated. A similar outcome has also been reported from Pakistan, Egypt and other countries[20-25].

There are studies from Bangladesh, India, South Korea and Turkey suggesting that HBeAg negative CHB patients as a whole tend to develop more significant liver fibrosis than those who are HBeAg positive[26-30].

An exact explanation for such a high incidence of HBeAg negative infection in our young chronic HBV infected population of the immunotolerant age group is difficult to reach. All 8 patients in our series, who were randomly picked up, tested negative for precore mutation. However, in Bangladesh most HBV infections are acquired early in life, either soon after birth or at a preschool age[1]. This possibly leads to early termination of an immune tolerance state in our population.

Non-alcoholic fatty liver disease (NAFLD) is now regarded as a leading cause of chronic liver disease in Bangladesh, perhaps second only to HBV infection. The incidence of non-alcoholic steatohepatitis (NASH) in our NAFLD patients is 88.5%[31]. Co-existence of NASH and CHB may also be responsible for significant hepatic injury in many of the apparently healthy chronic HBV infected population; however, this is an area that needs much more exploration. Finally, viral genotype may also be responsible[32].

Although many patients included in this present study were suitable candidates for anti-viral treatment, they are

usually not considered for treatment owing to complex socio-economic problems, social taboos and lack of scientific information. However, we recommend that all HBV-infected patients, irrespective of their age, should be properly evaluated for anti-HBV therapy.

Our study has a few limitations. One is that HBV DNA, ALT and liver histology were assessed only once. Serial assessment of virological, biochemical and histological parameters would provide more insight into the natural disease course in these patients. Our main aim was to gain an insight into the pathogenesis of these patients to initiate a strategy for their management. We found that a considerable number of young Bangladeshi HBV infected individuals have significant liver damage. This is important evidence to convince physicians and policy makers in developing countries to develop a management strategy for such patients.

In conclusion, chronic HBV infection is a complex disease entity and here we describe a group of such patients whose clinical course is not well studied and is difficult to explain. Although considered to be apparently healthy, a proportion of them are eligible for treatment. They not only pose a threat to themselves. In fact, in the fragile health economics of the developing world, they simply give rise to more questions than answers. As clinical hepatologists of the developing world, it remains our responsibility to look into these issues in further detail and develop a strategy for their appropriate management.

COMMENTS

Background

The immune tolerance phase usually persists for 20-25 years in chronic hepatitis B virus (HBV) infected subjects. However, early termination of the immune tolerance phase is seen in clinics.

Research frontiers

The clinical, biochemical, virological and histological aspects of young chronic HBV-infected patients of Bangladeshi origin were analyzed.

Innovations and breakthroughs

Early termination of the immune tolerance phase was detected in considerable numbers of chronic HBV-infected patients in this cohort. Many of them also developed progressive liver damage and increased fibrosis.

Applications

The management of these patients remains a major challenge as they express anti-HBe, a marker usually considered to have a better prognosis in the context of chronic HBV infection.

Terminology

Immune tolerance phase: HBV infected patients expressing hepatitis B e antigen, high HBV DNA but no liver damage.

Peer review

The article is properly written, endeavored and well constructed. Although there are an inadequate number of patients, it is an interesting article in terms of having insight on regional data.

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