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

Mamun AM *et al*. Immune tolerance state of hepatitis B virus

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**Abstract**

**AIM**: To assess whether this phenomenon is related to 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. Pre-core mutation (PCM) study were accomplishedin 8 patients who were randomly selected.

**RESULTS:** Forty four (53.7%) patients had significant necro-inflammation (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 (< 105 copies/mL) was observed in 57 (70.4%) patients. PCM was negative in all 8 patients.

**CONCLUSION:** This study indicates that thecurrent concept of age-related immune tolerance state of HBV infectiondeserves further analyses in different population groups.

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**Key word:** Chronic hepatitis B; Immune tolerance; Early termination

**Core tip:** Immune tolerance phase usually prevails 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 paucity of information about their entity.

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**INTRODUCTION**

May be nearly 2 billion people have been infected with hepatitis B virus (HBV) worldwide. The clinical manifestations vary widely with asymptomatic acute viral B hepatitis on one end and hepatocellular carcinoma (HCC) on the other end. There are about 400 million chronic HBV careers worldwide. Of them 75%-80% reside in Asia and Western Pacificregion. 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 paucity of information about nation-wide survey regarding HBV prevalence in Bangladesh, published data show that about 5%-6% apparently healthy individuals are HBV carrier 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]. 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]. It results in HBeAg negative HBV infection. Core promoter region (nucleotides 1742 to 1849) has 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 aninactive 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 at Bangladesh. It seems that there may be an early termination of immune tolerance state of HBV at Bangladesh. However, there is no therapeutic recommendation for these young HBV-infected patients. Here, we provided evidences suggesting that considerable numbers of these patients should be treated; otherwise they may develop complications of chronic HBV infection.

**MATERIALS AND METHODS**

***Patients***

One hundred 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 without any feature 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 minimum at 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 any antiviral drug for treatment of HBV infection.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 case of minors. Informed written consent for undergoing per-cutaneous liver biopsy was obtained. Patients were excluded from further analyses if adequate amount of liver tissues (*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 could not be available from them. Thus, a total of 81 HBeAg negative chronic hepatitis B (CHB) patients were included for final analyses.

***Biochemical and serological tests***

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 ELISA kit (Diasorin, Fallugia, Italy). HBeAg was checked in the sera using 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, USA). 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 DNA sample was heated at 95°C for 7 min to denature proteases, spun in a microcentrifuge for 5 s 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.), 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°C for 1 min, at 55°C for 1.5 min, and at 72°C for 3 min in each cycle. In the last cycle the reaction at 72°C 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, USA). Biopsy specimen of more than 1.0 cm in length with five to six portal tracts wasevaluated. Histology was graded according to histologic activity index (HAI) using the criteria of Knodellet. Al[17]. The total HAI score comprises necro-inflammation (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 *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 sufficient amount of liver biopsy specimens could be collected from these patients. The base line data of these patients is given in Table 1. Sixty of them (74%) were male and the rest 21 were female (26%). The numbers of male patients were significantly higher than female (60 *vs* 21, *P* < 0.05). The age of the patients were 17.5 ± 2.6 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 rest 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 × 1012 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 difference with patients with low and moderate hepatic necroinflammation (44 patients, *P* > 0.05). Significant levels of hepatic fibrosis (HAI-F ≥ 3) were detected in 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 necro-inflammation 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 immuno-tolerance age group tend to have no significant hepatic pathology.

Although the study shows that a significant proportion of our patients wereat 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 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 are indicated. 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 high incidence of HBeAg negative infection in our young chronic HBV infected population of the immuno-tolerant age group is difficult to reach. All the 8 patients in our series, who were randomly picked up, tested negative for pre-core mutation. However, in Bangladesh most HBV infections are acquired early in life either soon after birth or in pre-school age[1]. This possibly leads to early termination of 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 next 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 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 was 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 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 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 also difficult to explain. Although considered to be apparently healthy, a proportion of them are eligible for treatment. They not only pose threat to themselves. In fact, 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 details and develop strategy for their appropriate management.

**COMMENTS**

***Background***

Immune tolerance phase is usually persists until 20-25 years in chronic hepatitis B virus (HBV) infected subjects. However, early termination of 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 immune tolerance phase was detected in considerable numbers of chronic HBV-infected patients in this cohort. Many of them also developed progressive liver damages and increased fibrosis.

***Applications***

It remains a major challenge about management of these patients as they express anti-HBe, marker usually considered to have 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 damages

***Peer review***

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

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**Table 1 Baseline characteristics of study population**

|  |  |
| --- | --- |
| ParametersTotal number of patients | 81 |
| Male | 60a (74%) |
| Female | 21 (26%) |
| Age (in years) | 17.5±2.6 (12-20) |
| ALT ≤ 42 (U/L) | 52a (65.2%)[13-42] |
| ALT >42 (U/L) | 29 (34.8%) [44-281] |
| DNA ≤ 100000 (copies/mL) | 57a (70.4%) |
| DNA >100000 (copies/mL) | 24 (29.6%) |
| Non-significant hepatic necro-inflammation (HAI-NI ≤ 7) | 44 (53.8%) |
| Significant hepatic necro-inflammation (HAI-NI >7) | 37 (45.7%) |
| Non-significant hepatic fibrosis (HAI-F < 3) | 66a (81%) |
| Significant hepatic fibrosis (HAI-F ≥ 3) | 15 (18.5%) |
| Cirrhosis | 2/15 |

Figure in the round bracket indicates the percentage and that is square bracket indicates range. a*P* < 0.05 *vs* same parameter.