Original Article

Hepatitis B e Antigen Negative Chronic Hepatitis in Indian Patients : A Reality

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Abstract

Background: Hepatitis B e antigen negative chronic hepatitis (e^- CHB) with detectable levels of hepatitis B virus DNA (HBV DNA) in serum has been reported in cases from Asia. This study was undertaken to find out prevalence e^-CHB and to correlate its presence with the clinical status and severity of the illness in cases of chronic liver disease in India.

Methods: All patients of infective hepatitis, who were hepatitis B surface antigen (HBsAg) positive by enzyme-linked immunosorbent assay (ELISA), were evaluated with liver function tests and HBeAg and antiHBe antibody studies. Polymerase chain reaction (PCR) test was carried out to detect HBV DNA qualitatively.

Result: Out of 2064 samples tested by ELISA, 429 (20.78%) were HBsAg positive. HBV DNA (qualitative) was performed on all 429 patients and 74 (17.2%) were HBV DNA positive. Of these only 42 (56.75%) tested positive for HBeAg. Overall, 8.3% of HBeAg negative patients (32/384) were viraemic with evidence of chronic liver disease/clinical cirrhosis and alteration of transaminase levels, while three cases (0.84%) HBeAg positive cases did not show presence of HBV DNA.

Conclusion: This study shows e^-CHB prevalence rate of 8.3% in patients with HBV infection in India. Since HBeAg negative patients had detectable levels of HBV DNA as seen in HBeAg positive patients, benefit of antiviral therapy should be given to them. Population studies on e^- CHB cases are needed to determine its true prevalence, natural course and response to therapy.

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Key Words : Chronic hepatitis; Hepatitis B virus; Hepatitis B e antigen negative

Introduction

Hepatitis B virus (HBV) is a DNA virus which replicates asymmetrically via a reverse transcription of an RNA intermediate, making it prone to mutations in the genome at an approximate rate of one nucleotide/ 10,000 bases/infection year [1]. Hepatitis B e antigen is derived from the translation product of the precore and core regions. It is selected from the infected hepatocyte because of a secretory signal sequence at the beginning of the precore region [2].

Seroconversion from hepatitis B e antigen (HBeAg) to e antibody (anti-HBe) is usually accompanied by cessation of HBV replication and remission of liver disease [3]. Studies on the clinical implications of the variability of HBV genome have led to the identification of the most common naturally occurring HBV variants which include the precore (Pre-C) stop codon mutation ($G_{1896}A$) which abolishes hepatitis B e antigen (HBeAg) production and the dual mutation in the basal core promoter (BCP) region (A_{1762} T, G_{1764} A) which down regulates HBe antigen production [4]. These variants, particularly the precore variants are found in majority of patients with HBeAg negative chronic hepatitis, in which HBV replication and hepatic inflammation persists despite the absence of HBeAg. Precore and core promoter variants have been reported in upto 50- 80% of patients with HBeAg negative chronic hepatitis in Europe and Asia [5]. The phenomenon of HBeAg negative chronic hepatitis B (e^-CHB:- HBsAg positive, HBeAg negative, serum HBV DNA detected by polymerase chain reaction and with clinical /biochemical evidence of liver disease) was initially thought to be rare and largely confined to the Mediterranean basin, but as of now the prevalence of e^-CHB is considered to have surpassed that of e^+CHB in some of the Mediterranean countries [3]. This study was done up to find prevalence of hepatitis B e antigen negative cases of chronic hepatitis (e^-CHB) in India and to correlate it with their clinical status/severity of the liver disease. However due to non availability of the sequencing facilities, detection of the mutations in the precore or BCP regions could not be done.

Material and Methods

This cross sectional study of patients with HBV infection

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was conducted over a period of two years. Patients on antiviral therapy or co-infected with hepatitis C virus (HCV), hepatitis D virus (HDV) and human immunodeficiency virus (HIV) were excluded. Demographic data was compiled. HBsAg, HBeAg, HBV DNA and antiHBe antibody were the Hepatitis B markers included in the study. Serum albumin, bilirubin, aspartate transaminase, alanine transaminase (ALT) and alkaline phosphatase were the liver function tests included. HBsAg was detected by enzyme-linked immunosorbent assay (ELISA) manufactured by Ranbaxy Laboratories Limited and other serological markers by using commercially available ELISA kits (RADIM Italy).

The DNA extraction was performed using QIA amp blood, serum and body fluid kit (Qiagen) according to manufacturers instructions and put up for amplification. Amplification for HBV DNA was performed using primers to detect wild type and precore mutants [6].

Primer 1 (forward) 5'- CTG GGA GGA GTT GGG GGA GGA GAT T- 3'

Primer 2 (reverse) 5'- GGC GAG GGA GTT CTT CTT CTA GGG G -3'.

50µL polymerase chain reactions (PCR) were set up using 5ml 10X reaction buffer (10mM Tris-HCl pH 8.3 at 20^{0}C, 500mM KCl, 30mM $MgCl_2$), 0.5ml 10mM dNTPs(10mM of each A, C,G and T), 1 ml primer-1 (60ng/ml),1ml primer 2 (50ng/µl), 0.25ml (1.25units) Taq polymerase (Bangalore-Genei) and 32.25 ml double distilled water (DDW). Thermal cycling parameters were: 94°C x 5 minutes, 50°C x 1 minute, 72°C x 2 minutes (first cycle), followed by thirty cycles of 94°C x 1 minute, 50°C x 1 minute, 72°C x 2 minutes and a final cycle of 94°C x 1 minute, 50°C x 1 minute 72°C x 7 minutes. Amplified products were visualized after electrophoresis on 1.5 % agarose gel under ultraviolet light.

Results

The mean age of patients was 28 ± 1 year (range 3-67 years) and majority were males. Out of 429 HBsAg positive patients, majority 384(89.5%) were HBeAg negative, while 45 (10.5%) were HBeAg positive. Serum HBV DNA was detected in 74 (17.2%) patients, predominantly amongst HBeAg positive 42 (56.7%) patients, while 32 (43.2%) were HBeAg negative (Table 1). Of the 32 HBeAg negative patients with detectable HBV DNA, 24 were diagnosed as cases of chronic hepatitis and eight were healthy carriers. All the HBeAg negative patients were positive for anti-HBe antibody. 20 (62.5%) HBeAg negative patients had abnormal ALT levels, five (15.6%) had normal ALT and four (12.5%) had cirrhosis (Table 1). Thus a total of 24 (75%) of HBV DNA positive HBeAg negative cases had elevated ALT and /or cirrhosis. Out of 429 HBsAg positive patients tested for HBV DNA, 24 (5.5%) patients were HBeAg negative and HBV DNA positive with elevated ALT and/or cirrhosis.

A significantly lower proportion 32(43.2%) of HBeAg negative cases had detectable HBV DNA as compared to 42 (56.7%) of HBeAg positive patients. It was seen that 40 (95.2%) of HBeAg and HBV DNA positive patients, had abnormal ALT levels or cirrhosis, as compared to 24 (75%) of HBV DNA negative patients (Table 1). A strongly positive correlation was observed between HBeAg and HBV DNA positivity and 42 of the 45 (93.3 %) HBeAg positive patients had detectable levels of HBV DNA, whereas of the 384 HBeAg negative patients only 32 (8.3 %) tested positive for HBV DNA. Only three (6.7%) patients positive for HBeAg were negative for HBV DNA. In HBeAg negative or postive cases with HBV DNA, there was no significant derangement of prothrombin time and ALT levels in those with serum bilirubin levels of less than1.4mg/l.

Table 1

Comparitive profile of Hepatitis B e antigen negative and positive patients of chronic hepatitis

	HBeAg negative HBV DNA positive	HBeAg positive HBV DNA positive
Total positive cases of HBV DNA (n=74)	32 (43.2%)	42 (56.7%)
↑ ALT (IU/l)	20 (62.5%)	32 (76.19%)
Normal ALT	5 (15.6%)	6 (14.2%)
Cirrhosis	4 (12.5%)	8 (19.0%)
Total bilirubin	1.4 ± 0.1	1.0 ± 0.1
Prothrombin time	12.5 ± 0.01	12.6 ± 0.2

Discussion

Chronic hepatitis represents a series of liver disorders of varying causes and severity in which hepatic inflammation and necrosis continues for at least six months. It can be divided into two phases based on the relative level of HBV replication. The replicative phase, characterized by the presence of serum markers of HBV replication (HBeAg and HBV DNA), detectable intrahepatocyte nucleocapsid antigens (primarily hepatitis B core antigen HBcAg), high infectivity and liver injury. HBV DNA can be detected in the liver but it is extrachromosomal. In contrast, the nonreplicative phase is characterized by the absence of conventional markers of HBV replication (HBeAg and HBV DNA detectable by hybridization), an association with anti-HBe, absence of intrahepatocytic HBcAg, limited infectivity, and minimal liver injury. HBV DNA can be detected in the liver but is integrated into the host genome [7]. Patients in the replicative phase tend to have severe chronic hepatitis while those in the nonreplicative phase are asymptomatic hepatitis B carriers or have minimal disease. However, distinctions in HBV replication and histological category do not always coincide.

In the present study, HBV DNA was detected in 74 (17.2%) of 429 HBsAg positive patients. 384(89.5%) cases were HBeAg negative, while 45(10.5%) were HBeAg positive. Cirrhosis was detected in both category of cases showing presence of HBV DNA, with predominance amongst both HBV DNA and HBeAg positive cases at 19.0% as compared to 12.5% in HBeAg negative cases (Table 1).

Chronic HBeAg negative disease is known to be associated with either a continuous course of persistent increased ALT levels or a discontinuous course with multiple peaks of aminotransferase [8]. On comparing ALT levels, it was seen that 62.5% of HBeAg negative HBV DNA positive patients had abnormal enzyme levels as compared to 76.19% of HBeAg positive patients (Table 1).

During early chronic HBV infection, HBV DNA can be detected both in serum and in hepatocyte nuclei, where it is present in free or episomal form. This replicative stage of HBV infection is the time of maximal infectivity and liver injury. HBeAg is a qualitative marker and HBV DNA a quantitative marker of replicative phase, during which all three forms including intact virions of HBV circulate [9]. The replicative phase of chronic HBV infection gives way to a relatively nonreplicative phase at a rate of approximately 10% per year and is accompanied by seroconversion from HBeAg positive to anti-HBe positive. In most cases, this seroconversion coincides with a transient, acute hepatitis like elevation in aminotransferase activity reflecting cell mediated clearance of virus infected hepatocytes. In the nonreplicative phase of chronic infection, when HBV DNA is demonstrable in hepatocyte nuclei, it tends to be integrated into the host genome. In this phase, only spherical and tubular forms of HBV (not intact virions) circulate and liver injury tends to subside.

The phenomenon of HBeAg negative chronic hepatitis B (e^-CHB) was initially thought to be rare and confined largely to the Mediterranean basin, but studies from United Kingdom, Hongkong and India have shown a prevalence of 36 - 61% [10]. In this study, 43.2% of the cases who were positive for HBV DNA in the serum were negative for HBeAg (Table 1). Such patients usually have severe chronic HBV infection and detectable HBV DNA but with anti-HBe instead of HBeAg. Chang et al [11], in their study showed that a significant drop in HBV DNA was required for HBeAg seroconversion. An HBV DNA level of less than 10^4 copies/ml was predictive of inactive liver disease among patients undergoing HBeAg seroconversion and in HBeAg negative patients. These patients were found to be infected with an HBV mutant that contained an alteration in the precore region rendering the virus incapable of encoding HBeAg. Although several potential mutation sites exist in the pre-C region, the region of the C gene necessary for the expression of HBeAg is most commonly affected. Another mutation in the core promoter region prevents transcription of the coding region for HBeAg and yields an HBeAg-negative phenotype. Patients with such precore mutants that are unable to secrete HBeAg tend to have severe liver disease that progresses rapidly to cirrhosis and it does not respond readily to antiviral therapy.

Both wild type HBV and precore mutant HBV can coexist in the same patient or mutant HBV may arise during wild type HBV infection. The relationship between precore/core promoter variants, serum HBV DNA levels and severity of liver disease is unclear. In this study, three cases which were HBeAg positive were negative for HBV DNA(Table1). Seef et al [12], found that of the 1,171 cases which were HBeAg positive carriers, 13% were negative for HBV DNA where as out of the 1,362 anti-HBe positive carriers only 20% were HBV DNA positive by hybridization. Some in-vitro studies suggest that core promoter mutations increase HBV replication [13]. Another study based on PCR assay found that among patients with severe HBe Ag negative hepatitis, those with precore variants had higher HBV DNA levels as compared to patients with wild type (WT0) precore sequence [14]. Although, some studies have shown that these variants can be found in hepatitis B surface antigen carriers who have inactive liver disease [15].

With an estimated prevalence of HBsAg carrier rate of 4.7% and 80% of chronic liver disease patients having HBV infection in India [16], it is not surprising that a study from India of 363 consecutive patients of chronic HBV hepatitis detected 141(39%) to be HBeAg positive, while 222(61%) were HBeAg negative [10]. In the same study HBV DNA positivity was seen in 53 (40%) of 131 HBeAg negative patients. In another study, detection of pre-C mutants in 13.6% and 12.5% cases of acute and fulminant hepatitis B respectively was seen in India [17]. In this study of the 384 HBeAg negative patients, 102(26.5%) had clinical/biochemical evidence of liver disease and 32 (29.6%) of them had detectable serum HBV DNA. In this study 8.3% of all HBeAg negative patients had fulfilled the definition of e^-CHB. A higher prevalence rate of e^-CHB at 20% in Hongkong by Chan et al [3] and 18% in Mumbai has been reported by Amrapurkar et al [10]. However this may not be a true estimate of its prevalence, because of fluctuating ALT levels and /or intermittent detection of serum HBV DNA by PCR. In addition some HBeAg negative patients may have active liver disease on histology despite normal ALT and undetectable serum HBV DNA, especially they were assessed on one occasion only. In another study, it was shown that precore variants were predominantly detected in HBeAg negative patients, where as core promoter variants were found in both HBeAg negative and HBeAg positive patients [18]. This happens because precore variants abolish, where as core promoter variants only down regulate HBeAg production [9]. Lindh et al [14], have also reported an

association between serum HBV DNA level, histological inflammation, fibrosis scores and ALT values in HBeAg negative but not in HBeAg positive patients. This suggest that mechanisms of liver damage may be different in chronic hepatitis, with host immune response being more important in HBeAg positive patients and direct viral effects in HBeAg negative cases.

One of the important outcomes of HBV DNA testing has been the identification of patients who are HBsAg, anti-HBe positive but positive for HBV DNA. Such a clinical situation is associated with variations in ongoing chronic disease, fulminant acute hepatitis, acute non-fulminant hepatitis and poor response to interferon therapy. The underlying reason for their failure to clear HBV after initial infection is also probably linked to their inability to respond to interferon. The putative evidence of the presence of these precore mutants in HBeAg negative patients to the tune of 8.3 % (32/384) emphasizes the importance of this particular subset of chronic hepatitis patients.

Detailed population studies are necessary to determine true prevalence, natural course and response to therapy of e^-CHB. This study has shown that the overall prevalence of 8.3% of HBeAg negative chronic hepatitis, is not uncommon in India and chronic liver disease occurs in a significant number of them. A patient of chronic hepatitis who is HBeAg negative must be evaluated for detectable HBV DNA levels and the benefit of antiviral therapy could be given to such patients.

Conflicts of Interest

None identified

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