Infrequent occurrence of silent HBV infection among Indian patients with chronic liver disease

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Objective: To investigate the occurrence of silent hepatitis B virus (HBV) infection among patients with chronic liver disease (CLD). Methods: Plasma samples from 71 CLD patients including 9 HBsAg-positive individuals were tested for HBV DNA by nested polymerase chain reaction (nPCR), and for HBV serum markers, i.e., anti-HBc antibody, HBsAg and anti-HBe antibody. The individuals were also tested for hepatitis C virus (HCV) RNA and anti-HCV antibody. Results: Among 62 HBsAg-negative patients, silent HBV infection was seen in only two (3.2%). Silent HBV infection was not found in any of the 26 patients who had evidence of HCV infection. One HBsAg-positive patient was positive for anti-HCV in the absence of HCV RNA. Conclusions: There is a low rate of silent HBV infection among patients with CLD in India, where HBV is moderately endemic. Silent HBV infection is not associated with HCV-related CLD, which is in contrast to reports from other HBV-endemic areas in Asia. [Indian J Gastroenterol 2001;20:87-89]

Key words: HBV DNA, HCV RNA

Hepatitis B virus (HBV) is an important cause of chronic liver disease (CLD) in India. The most important primary diagnostic test detects the presence of hepatitis B surface antigen (HBsAg). With the development of sensitive molecular techniques like nested polymerase chain reaction (nPCR), serum HBV DNA has been detected in the absence of HBsAg among blood donors and healthy individuals, sometimes in the presence of antibodies to the core and surface antigens.1,2,3

HBsAg-negative HBV infection detected by presence of serum HBV DNA has also been reported in CLD.4,5,6 Such infection has been referred to as occult, cryptic, inapparent or silent HBV infection.7,8,9 Occurrence of such infection has been attributed to the presence of mutations in the surface gene leading to HBsAg negativity or to mutations in the X region of the HBV genome resulting in low-level HBV replication.6,8 More recently, silent HBV infection is increasingly being reported in patients with hepatitis C virus (HCV)-related CLD residing in regions where HBV is endemic, with prevalence rates ranging from 50% to 87%.8,10 In such co-infections, silent HBV has been implicated in the resistance of HCV to interferon therapy.9 In vitro cotransfection studies have shown that the silent HBV promotes HCV replication as seen by increased RNA levels compared to coinfected wild-type HBV.8

We investigated CLD patients including those with established HCV-related disease for the occurrence of silent HBV infection, defined as presence of serum HBV DNA as detected by PCR in the absence of HBsAg in individuals who may or may not be anti-HBc positive.10

Methods

Seventy-one non-consecutive patients with CLD of suspected viral etiology, who presented to the out-patient services of the Department of Gastrointestinal Sciences, were included in the study. Patients with Wilson’s disease, hemochromatosis, primary biliary cirrhosis and those on interferon treatment were excluded. Among the patients studied, 62 were HBsAg-negative and 9 were HBsAg-positive. Patients were diagnosed to have CLD based on history of illness for longer than 6 months, clinical findings and laboratory investigations. Clinical diagnosis of chronic hepatitis (n=14) was confirmed on histology, and cirrhosis (n=44) by ultrasound findings and on histology when ultrasound findings were not definitive. Patients in whom differentiation into cirrhosis or chronic hepatitis was not possible sonographically and liver biopsy was not done were labelled as CLD-unclassified (n=13).

The control group (n=50) consisted of 39 HBsAg and anti-HCV negative patients attending the out-patient clinic for endoscopy, having normal liver enzymes, and 11 healthy HBV-immunized laboratory personnel.

HBV PCR

HBV DNA was extracted using Qiaamp blood kit (Qiagen, Germany), according to the manufacturer’s instructions, from 200 mL of freshly thawed plasma that had been stored at -60°C. Extracted DNA was amplified with two separate sets of primers specific for the surface (outer: S5 and S6; inner: 109 and 585R) and core (outer: 1763 and 2032; inner: 1788E and 2017R-B) genes of the HBV genome.11,12 Detection of amplified products from one or both regions was considered positive.
The amplification was carried out in a 50 mL reaction volume containing 10 mL of extracted DNA, 1 unit of Taq polymerase, 0.2 mM of dNTP mix, 50 mM KCl, 10 mM Tris-HCl, 3mM MgCl₂ (Boehringer Mannheim- Roche, GmbH, Germany) and 20 pmols of each outer primer pair (surface or core-gene specific) [Gibco BRL, UK]. The mixture was heated to 95°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1.5 min, primer annealing at 55°C for 1.5 min and extension at 72°C for 2 min, followed by a final extension at 72°C for 10 min. The annealing temperature was modified from the protocol followed by Kaneko et al, from 42°C to 55°C.11

The nested PCR step was carried out with similar protocol with 5 mL of the amplified product of the first round PCR and inner primer pairs (surface or core gene specific). Second round PCR products were subjected to 2.5% agarose gel (Genei, India) electrophoresis and ethidium bromide (0.5 mg/mL) staining and the band of interest identified using UV light transillumination (Mighty Bright: Hoefer, USA). Phi X174 Hae III digest (Genei, India) was used as the molecular weight standard for each assay. The size of the amplified surface gene product was 477 bp and that of core gene product was 258 bp. The nPCR for the core region has been shown in our laboratory to compare well with a commercial assay.13

HCV RNA was detected by reverse transcriptase-PER using a nested PCR as described previously.14

Serology
All 71 patients were tested for anti-HBe (total), HBeAg and anti-HBe (Abbott Laboratories, IL, USA). Anti-HCV antibody was tested using UBI HCV EIA 4.0 (United Biomedical, NY, USA) or IMA / Axsym HCV version 3 (Abbott laboratories, IL, USA).

Results
All nine HBSAg-positive patients tested positive for HBV DNA (Table). Among the 62 HBSAg-negative individuals, 38 were positive for anti-HBe. Two (3.2%) of them (both with cirrhosis) were HBV DNA positive; both these were also anti-HBe positive and one was anti-HBe positive. All HBV DNA-positive samples amplified both regions of the genome, except one of the silent HBV patients in whom PCR yielded amplification of the surface gene only. None of the 50 control subjects was HBV DNA positive. Four of the HBSAg-positive individuals were also positive for HBeAg; the other five were anti-HBe positive. Anti-HBe was also positive in two patients along with HBeAg.

Among the 62 HBSAg-negative patients, HCV-related disease was established by the presence of HCV RNA and/or anti-HCV in 26 patients. Silent HBV was not found in any patient with HCV-related infection.

| Table: HBV and HCV markers in CLD patients (n=71) |
|---------------------------------|------------------|------------------|
| Virus marker                   | HBSAg positive   | HBSAg negative   |
|                                | (n=9)            | (n=62)           |
| HBV DNA+                       | 0                | 2                |
| HBeAg+                         | 4                | 0                |
| Anti-HBe+                      | 7                | 16               |
| HCV RNA+ alone                 | 0                | 0                |
| Anti-HCV+ alone                | 1                | 1                |
| HCV RNA+, anti-HCV+            | 0                | 9                |

One HBSAg-positive individual was also infected with HCV (dual infection), being positive for anti-HCV alone. Anti-HBe positivity in the absence of other markers of HBV infection was seen in one patient who was also positive for HCV RNA in the absence of anti-HCV.

Discussion
The frequency of silent HBV infection among our CLD patients was low; it was seen in only 2 (3.2%) of the 62 HBSAg-negative CLD patients, both of whom had cirrhosis. None of the 26 HCV-infected CLD patients had silent HBV infection. This is in contrast to reports from other HBV-endemic regions of Asia, like Taiwan, China and Japan, where 50% to 87% of HCV-infected CLD patients had silent HBV infection.10 These Far Eastern countries have a high prevalence of HBV, whereas India has only an intermediate prevalence. HCV infection seems to have a similar prevalence in India and the Far East. Our findings may thus reflect the lower endemicity of HBV in our population. Alternatively, such variations may also depend on racial differences.

The presence of HBV DNA in the absence of HBSAg has been studied previously in Indian patients by Gupta et al14 in their study of HBV mutants. Of their 120 patients with HBV-related CLD, excluding HCV-infected patients, 10.8% were positive for HBV DNA and negative for HBSAg, suggesting the presence of surface mutants. They also reported a frequency of 15.5% precore mutation associated with absence of HBeAg. The five HBSAg-positive patients in our study who were anti-HBe positive and HBeAg negative but HBV DNA positive may be harboring precore mutants; however, this needs to be confirmed by genomic sequence studies. The presence of anti-HBe in HBsAg and serum HBV DNA negative but anti-HBe positive patients may be an indication that HBV is present in the liver; this needs investigation of liver biopsy material. Sole anti-HBe positivity (in the absence of all other HBV markers) in the one HCV RNA positive patient was probably a false positive reaction; such a phenomenon is rare following HBV infection.

In summary, there is a low occurrence of silent HBV infection among HBSAg-negative CLD patients in our study population. The virological investigation of
CLD should include: HBV DNA testing when silent HBV infection is suspected. Detecting serologically silent HBV infection among patients with CLD is especially important in those undergoing liver transplantation and those being treated for presumed autoimmune hepatitis since positivity of autoimmune markers may be related to HBV infection.

References

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