ORIGINAL ARTICLE

Occult Hepatitis B as a Cause of Cryptogenic Cirrhosis

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ABSTRACT

Background: Occult hepatitis B virus (HBV) infection is characterized by presence of HBV infection with undetectable hepatitis B surface antigen (HBsAg). Diagnosis of occult HBV infection requires sensitive HBV-DNA PCR assay. Recently it has been shown that occult hepatitis B may be a cause of cryptogenic liver disease. The aim of this study is the investigation of occult HBV infection among patients with cryptogenic liver disease.

Methods: 65 consecutive paraffin-embedded liver tissues from cases referred to RCGLD (Research Center for Gastroenterology and Liver Diseases) and THC (Tehran Hepatitis Center) during the years 2001 and 2002 for liver biopsy because of elevation of alanine aminotransferase (ALT) levels for more than six months were studied. Among these, 12 patients with cryptogenic liver disease were found. Human tissue DNA could be extracted in 7 of 12 patients. In these patients liver biopsies were reviewed and HBV-DNA and HBsAg and HBcAg were assayed in liver tissue by polymerase chain reaction (PCR) and immunohistochemistry (IHC), respectively.

Results: Histologically, chronic hepatitis, cirrhosis and nonspecific changes were reported. HBVDNA was detectable in 4 patients but IHC was negative in all. The frequency of occult HBV infection was more than 50%.

Conclusions: Occult HBV infection is common among patients with cryptogenic liver disease. In these patients, HBV-DNA may be detected more frequently among

patients with more advanced liver pathology (cirrhosis) and more aggressive clinical course (decompensated cirrhosis).

Key Words: HBV, Chronic Hepatitis, HBV-DNA, Cryptogenic Cirrhosis

INTRODUCTION

The proportion of patients suffering from liver disease of unknown cause ranges from 5% in chronic hepatitis to up to 40% in fulminant hepatitis cases. These patients may develop severe liver injury, leading to an increased risk of cirrhosis and liver cancer. Several studies have called attention to hepatitis B virus infection in the absence of serological markers or in the presence of anti-HBc alone. It has been demonstrated that the serum of some patients without detectable HBsAg may contain infectious virus. Accumulated data indicated that a low level of HBV-DNA remains detectable in serum and liver tissue in some patients who cleared HBsAg from either acute self-limited or chronic HBV infection (1-8). The frequency of HBV-DNA in patients with cryptogenic chronic liver disease varies depending on the baseline prevalence of HBV infection in certain geographical area, population studied, and techniques used to detect HBV-DNA. For instance, the prevalence of serum HBV-DNA as assessed by HBV PCR was 10.8% in patients with chronic hepatitis, but without HBV and HCV markers in India. One study performed on patients with 'cryptogenic' chronic hepatitis found that one third of them had detectable HBV-DNA, indicating an occult HBV infection. During follow-up, repeated liver biopsy demonstrated that about one fifth had progressed from chronic hepatitis to cirrhosis. These findings indicate that occult HBV infection is a common etiology of 'cryptogenic' chronic hepatitis and a progressive disease at least in some patients (1-5). This study was designed to determine the role of occult hepatitis B as an etiology of cryptogenic liver disease in Iran.

MATERIAL AND METHODS

Patients: During the years 2001-2002, 65 patients with chronic liver disease were investigated by Research Center for Gastroenterology and Liver Diseases (RCGLD) and Tehran Hepatitis Center(THC). From these, 12 patients with cryptogenic liver disease were obtained. All of these patients were negative for serum HBsAg and had been undergone liver biopsy. The paraffin-embedded liver tissue of these patients was investigated for presence of HLA-DR (human genome (and HBV-DNA (viral genome) by PCR, and existence of HBs-Ag and HBcAg by Immunohistochemistry (IHC) technique. All laboratory procedure (PCR and IHC) were performed in Iranian Blood Transfusion Organization (IBTO) laboratory with high technology and precession.

The patients were identified as cases with a persistent increase in alanine aminotransferase (ALT) (>1.5 time the upper limit of normal on at least two different occasions) or had impaired liver function over a minimum period of six months. Epidemiological data (previous history of acute hepatitis, transfusion, intravenous drug addiction, acupuncture, tattooing, etc), clinical data (patient's age, sex, body mass index and biochemical parameters including glucose, triglyceride, cholesterol, and protein electrophoresis) were obtained from patient's charts and recorded in questionnaire.

The cases met the following criteria 1.absence of HBsAg and anti-HCV antibodies in serum; 2.ferritin, alpha1 antitrypsin, and ceruloplasmin levels within the normal range 3. antinuclear, antimitochondrial, and anti smooth muscle antibodies at a titer of <1/40 4.ethanol intake less than 80 g/day; 5. absence of treatment with potentially hepatotoxic drugs; 6. negative human immunodeficiency virus (HIV) serology; 7. absence of decompensated diabetes, thyroid dysfunction, morbid obesity (defined as body mass index >35 kg/m2) or other systemic diseases which might affect the liver including severe hyperlipidemia (cholesterol or triglyceride serum levels greater than 350 mg/dl) and 7. absence of focal intrahepatic lesions or biliary abnormalities at ultrasonography or even normal endoscopic retrograde cholangiopancreatography (ERCP) in some patients.

Liver histopathology: Reported histological parameters included: fibrosis, portal inflammation, piecemeal necrosis, lobular inflammation, lobular necrosis, cholestasis, bile duct damage, sinusoidal dilation, and iron deposition. According to these data, liver biopsies were classified as follows: chronic hepatitis and cirrhosis according to international criteria (9); non-specific/minimal changes: a variety of mild abnormalities including intrahepatocytic cholestasis, steatosis, sinusoidal dilation, and mild lobular inflammation or necrosis.

Immunohistochemistry: Immunoperoxidase staining for HBV surface and core proteins was performed by kits from DAKO company (clone HBc Ag, lot No: 128, antibody concentration: 1/500 and code No: BO586 and clone HBsAg: 3E7, lot No: 058, antibody concentration: 1/50and code No: M3506) in all liver samples. Then samples were interpreted by immunnofluorecent microscope.

HBV-DNA PCR: DNA was extracted from paraffin-embedded liver tissue using Greer CE method (10) with some modification. A fundamental safeguard to prevent cross contamination between block preparations was used. Every extraction and PCR set included: 12 sample, seven paraffin-embedded blocks, three water samples as negative controls to detect cross contamination and two positive controls to establish sensitivity, corresponding 3000 and 300 geq/ml (VQC Panel) PCR was performed using the following primers: Primer#1 (nt109-139) ATACCACAGAGTCTAGACTCGTGGTGGACT. Primer 2R (nt 555-586) AAGCCCCTACGAACCACTGAACAAATGGCAC. Briefly, 6µl DNA was amplified for 35 cycle 95C for 1min, 60C for 1min and 72C for 1min followed by final extension at 72C for 10 min. In total reaction mixture of 20µl containing 10 mM Tris-Hcl PH8.3, 1.5 mM Mgcl2 (promega) 50 mM Kcl 200µM dNTP (Roche) 1.5 U of Taq-polymerase and 0.5 µM each primer. 10µl of reaction mixture were loaded on a 2% agarose gel electrophoresis. All precautions for avoiding contamination were followed stringently. Each positive result was confirmed by a second independent determination. The sensitivity of assay was 300 gem/ ml. Occult hepatitis B was defined as detectable HBV-DNA in liver tissue.

Statistical analysis: Fisher's exact test was used to compare proportions and the Student's t test to compare continuous variables. The Mann-Whitney U test was used to compare non-parametric variables in independent samples.

All statistical tests were two tailed. Correlations between the variables were calculated using Spearman rank order correlations. A P value of 0.05 (two-tailed) was considered to indicate significance.

RESULTS

Patients: We evaluated 65 patients referred for liver biopsy because of persistent (more than six months) elevation in serum transaminases. According to clinical, biochemical, serological and pathological testing following results were obtained: hepatitis C in 45 cases, cryptogenic liver disease in 12 cases, NASH in 1 case, autoimmune liver disease in 3 cases, hepatocellular carcinoma in 2 cases, primary biliary cirrhosis in 1 case, metastatic adenocarcinoma in 1 case. To make sure the detection of human genome and to prevent false negative result for HBV-DNA, all cases with undetectable HLA-DR (not enough tissue) were excluded (5 cases). So, our cryptogenic liver disease cases decreased to 7; that is 3 males (42.9%) and 4 females (57.1%) with mean age of 43(±13.55) were studied. Most of the patients presented with non-specific symptoms 3 (42.9%) or clinical manifestation of the chronic hepatitis 2 (28.6%).

Signs and symptoms of advanced liver disease or cirrhosis had been reported in 2 (42.9%) of patients.

Clinical and Biochemical findings according to result of HBV-DNA PCR:

According to result of HBV-DNA PCR, the patients were divided to two groups, HBV-DNA positive and HBV-DNA negative. (table1) Clinical and biochemical findings were compared between two groups. As shown in table 1, no significant differences were found between two groups according to age, sex, duration of disease, history of risk factors (transfusion, IVDs, needlestick or surgery), clinical diagnosis and level of aminotransferase. Although not statistically significant, decompensated cirrhosis and more advanced pathology were found in HBV-DNA positive group.

Viral markers and immunohistochemistry:

HBsAb was reported to be negative in all cases. HBcAb in 1 case (14.3%) was positive. None of HBV-DNA+ patients were positive for HBcAb. No significant correlation was found between HBV-DNA positivity and presence of HBcAb or HBs Ab in serum. HBs and HBc proteins were investigated in liver biopsies by immunohistochemistry.

All cases were found to be negative. Of 7 patients with cryptogenic liver disease, 4 patients (57%) were positive and 3 (43%) patients were negative for HBV-DNA. Symptoms of cirrhosis were more prevalent in the group of patients with detectable HBV-DNA than in cases with negative HBV-DNA. The prevalence of HBV-DNA in the different histopathological forms of liver damage is shown in table1.

Table 1: Clinical , biochemical and histological features of patients with cryptogenic liver disease according to the presence of HBV-DNA

	HBV-DNA+ (N=4)	HBA DNA- (N=3)	P Value
Age(Y)	44± 16	41±11	0.806
Sex (M/F)	2:2	1:2	1.000*
Duration of Disease(M)	32±44	16±9	0.563
Risk factor			
Transfusion	1(25)	2(66)	0.486
IVDs	1(25)	0 (0)	0.350
Needlestick	0 (0)	1(33)	0.388
Surgery	3(75)	2(66)	0.405
Clinical Presentation			0.118
Decompensated Cirrhosis	2(50)	0 (0)	
Non Specific Symptoms	2(50)	3(100)	
Biochemical			
AST	146.±88	275±92	0.106
ALT	521±258	182±205	0.226
ALP	609±361	308±92	0.136
Pathology			
Cirrhosis	2(50)	0 (0)	
Chronic Hepatitis	0 (0)	2(66)	
Intrahepatic cholestasis	1 (25)	0 (0)	
Nonspecific	1 (25)	1(33)	

Values are mean (SD) or number (%).

*Fisher's exact test

DISCUSSION

In our case series, 18% of the patients were labeled as cryptogenic liver disease. In patients who underwent liver biopsy because of persistent alteration of liver biochemistry, the etiology of the liver lesion could not be determined from clinical, biochemical, or serological data obtained prior to the biopsy in 10% of cases. This value is similar to the reported prevalence of 9.2% and 8.2% for cryptogenic hypertransaminasemia observed in other European series (1, 11-13).

Frequency of occult hepatitis B in our patients was more than 50%. In another study up to 37% of patients who were labeled as having cryptogenic liver cirrhosis had occult HBV infection (4). Different reports have indicated that low level replication of HBV and/or HCV may play a pathogenic role in a proportion of cases with cryptogenic hepatitis or cirrhosis (1, 12, 13, 14).

Our study has demonstrated that occult HBV infection is common among patients with cryptogenic liver cirrhosis in an area where HBV infection is prevalent. HBsAg negativity per se is insufficient for exclusion of HBV infection, and screening for occult HBV infection by HBV-DNA assay is necessary before diagnosis of cryptogenic cirrhosis. Identification of occult HBV infection before, and prophylactic antiviral therapy after liver transplantation should be considered to prevent re-infection of the graft by HBV. Identification of HBV-related cirrhosis and surveillance for early, potentially resectable hepatocellular carcinoma by regular fetoprotein testing and/or ultrasound screening, and special precautions before accepting donations from patients with unexplained cirrhosis and atypical serological markers maybe beneficial (1, 4, 14).

Although not statistically significant, our study demonstrated that the patients with positive HBV-DNA had a higher prevalence of cirrhosis and also signs of decompensated cirrhosis more frequently than those with undetectable HB VDNA. These data are in accordance with reports showing a more aggressive course in cryptogenic cirrhosis when viral sequences can be detected in serum compared with patients who tested negative (1, 13, 18). In one study, the PCR methodology to detect an intact direct repeat region could amplify the ccc HBV-DNA, but not the incomplete HBV-DNA and integrated HBV-DNA, and showed ongoing occult HBV infection and more advanced liver pathology (8). None of our patients with occult HBV infection had serological markers of previous exposure to HBV. Another study, performed among Israeli patients, demonstrated that 30% of HBsAg negative patients with chronic liver disease had HBV-DNA detectable by PCR in serum (4). In that study, the positive rates of anti-HBc and anti-HBs were about 45%. Similar to the direct detection of HBV-DNA, serological markers of past HBV infection are also frequently detectable in HBsAg negative patients with chronic hepatitis C (from 20% to 55%) (7).

Immunoperoxidase staining for HBV surface and core proteins was negative in all the liver-biopsy specimens examined. These data suggest that occult HBV infection usually causes strong suppression of viral replication and gene expression (3, 19, 20).Viral or host factors allowing HBV persistence in the absence of HBsAg include: viral interference in co-infection with HCV or a new viral agent, HBV mutations in the core promotor region leading to minimal HBV replication and rearrangements or mutations in the HBsAg-encoding region of the viral genome, particularly in the S gene (16). It is also possible that in some cases host immune mechanisms can maintain HBV infection in a latent state until transmission to another individual who subsequently develops a more active infection especially when immunosuppressive therapy is employed (1-4, 8).Thus, serological recovery from chronic hepatitis B, marked by the absence of HBsAg, the occurrence of anti HBs, reductions in anti HBc titers and serum HBV-DNA clearance as measured by PCR, does not indicate HBV eradication and seems to merely represent immune control of viral replication. A replication-competent state of HBV infection might persist in the liver over many years after serological resolution (1, 8).

The outcomes of the occult HBV infection after a delayed HBsAg clearance vary significantly and may depend on the duration of active HBV infection and extent of liver injury that had occurred before HBsAg clearance and interval from HBsAg clearance to the time of assessment.

In conclusion, extensive studies have demonstrated that occult HBV infection represents a special form of HBV infection with clinical relevance. It seems to be common among patients with chronic liver diseases especially cryptogenic liver disease in Iran and more investigation with a larger number of patients is seriously needed in future.

ACKNOWLEDGEMENTS

This study has been funded by the Research Center of Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences. The authors wish to thank the staff of the Iranian Blood Transfusion Organization, and Tehran Hepatitis Center for their kind cooperation.

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