Occult Hepatitis B Virus Related Decompensated Cirrhosis of Liver in Young Males: First Report of Two Cases from Bangladesh

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Cryptogenic cirrhosis is a diagnosis of exclusion. Polymerase chain reaction (PCR) has demonstrated persistent hepatitis B virus (HBV) infection in serum and liver tissue of HBsAg-negative chronic hepatitis, HBsAg-negative cirrhosis, and HBsAg-negative HCC patients. The entity of occult HBV infection is well established. We report two patients with occult HBV related decompensated cirrhosis of liver for the first time from Bangladesh. The first patient is a young male with jaundice and hepatosplenomegaly. The second patient is also a young male with ascites. Both had altered liver function tests. Diagnosis of decompensated cirrhosis of liver was established in both cases and in both the etiology was identified by PCR to be occult HBV infection. In areas with high prevalence of HBV, a diagnosis of "cryptogenic" cirrhosis based on HBsAg testing alone is not adequate. The so called "cryptogenic" but actually occult HBV cirrhotics are suitable candidates for antiviral treatment. Occult HBV infection must be considered in all patients with cryptogenic cirrhosis of liver in areas where HBV infection is prevalent.

Keywords: Hepatitis B Virus, Occult Infection, Decompensated Cirrhosis, Bangladesh

Introduction

Over 350 million people worldwide are infected with hepatitis B virus (HBV) and globally around 1 million die due to consequences of this infection annually (1). Bangladesh belongs to the intermediate prevalence region for HBV infection. Here the lifetime risk of acquiring HBV is between 20% to 60% (2). Studies have shown that HBV is responsible for 31.25% cases of acute hepatitis (unpublished data), 76.3% cases of chronic hepatitis (3), 61.15% cases of cirrhosis of liver (4) and 33.3% cases of hepatocellular carcinoma (HCC) (5) in Bangladesh.

It has been observed that the most prevalent HBV genotype in Bangladesh is D (49%) followed by C (38%). In Bangladesh, patients with genotype C more often have serum ALT and AST elevation than those with genotype D. Also, HBV-DNA level is high in patients with genotype C (88%) compared to genotype D (32%). The number of positive patients for HBsAg is equal among those infected with the two genotypes. HAI tend to be higher in patients with genotype C infection (6). Limited data is available on the comparison of severity of liver disease between genotypes C and D infections. We found an association between genotype C and elevation of transaminase levels and higher viral load. A study from southern India showed that genotype C was associated with higher serum ALT levels than genotype D (7). In addition, subjects infected with genotype C virus tended to have higher HAI than those with genotype D. In a Japanese study, HBeAg positivity was more common in genotype C infections than in those with genotype D (8).

Cryptogenic cirrhosis is diagnosed by excluding all possible identifiable underlying conditions...
including viral, alcoholic, autoimmune and metabolic liver diseases. HBV infection can persist even after the loss of hepatitis B surface antigen (HBsAg). Such patients escape detection by conventional serological tests. Polymerase chain reaction (PCR) has demonstrated persistent HBV infection in serum and liver tissue of HBsAg-negative chronic hepatitis (9), HBsAg-negative cirrhosis (10, 11) and HBsAg-negative HCC patients (12). Residual HBV infection has also been detected in patients who had remission of HBV-related hepatitis and clearance of HBsAg either spontaneously or as a result of antiviral therapy (13-18). Such phenomenon is particularly seen in areas where HBV infection is prevalent and therefore a substantial number of HBV-related liver diseases may be missed if HBsAg alone is used for the diagnosis in such areas.

Case Report

Our first patient, a 19 year old young male, referred to us with jaundice. He was a higher secondary student from an upper middle class family. He was a non-smoker and non-alcoholic. He was non-diabetic and normotensive. He stayed in brick building, drank boiled water and used sanitary latrine. On examination, the patient had jaundice and hepato-splenomegaly, but no stigmata of cirrhosis. His investigation reports showed the following: total cholesterol=180 mg/dl, LDL=125 mg/dl, triglyceride=145 mg/dl, serum alanine transaminase (ALT)=40 mg/dl, His serum bilirubin, serum alanine transaminase (ALT), serum alkaline phosphatase, serum albumin and prothrombin time was 82 µmol/L, 71 IU/L, 64 IU/L, 185 IU/L, 35 gm/L and 16 sec. (control: 12 sec.), respectively. Ultrasonography of abdomen revealed hepatosplenomegaly. No space-occupying lesion was detected and serum alpha fetoprotein was 50.4 ng/ml. Endoscopy of upper gastrointestinal tract was normal. Anti-HCV, anti-HBs and anti-HBc were all negative, but HBV-DNA became detectable by PCR (Amplicon HBV Monitor Assay, RT-PCR, Roche Molecular Systems, California), which was positive with viral DNA load being 8×10⁷ copies/ml. The test was repeated and revealed similar results. We thus concluded that our patient was suffering from seropositive occult HBV related decompensated cirrhosis of liver with grade II esophageal varices due to portal hypertension.

Our second patient, another young, unmarried male university student aged 20 years, who was a non-alcoholic, but a smoker came to us with sudden onset of ascites. He came from a middle-class socio-economic background living in a brick building, drinking boiled water and using sanitary latrine. He had ascites on examination as well as the stigmata of cirrhosis. His investigation reports showed the following results: serum bilirubin=41 mol/L, serum ALT=131 IU/L, serum AST=194 IU/L, serum alkaline phosphatase=125 IU/L, serum albumin=20 gm/L and prothrombin time=12.5 sec. (control: 12 sec.), urinary copper<0.03 mg/L and ascitic fluid albumin=3 gm/L. KF ring was absent on ophthalmic examination. Abdominal ultrasonography showed coarse hepatic ecotexture. No space-occupying lesion was detected and serum alpha fetoprotein was 50.4 ng/ml. Endoscopy of upper gastrointestinal tract was normal. Anti-HCV, anti-HBs and anti-HBc were all negative, but HBV-DNA became detectable by PCR (Amplicon HBV Monitor Assay, RT-PCR, Roche Molecular Systems, California), which was positive with viral DNA load being 8×10⁷ copies/ml. The test was repeated and remained positive. We thus labeled the patient as a case of seronegative occult HBV related decompensated cirrhosis of liver.

Anti-HDV was not done in any of the above cases, as the test is unavailable in Bangladesh. Both patients were treated with combination oral antivirals, namely lamivudine (100 mg) daily and adefovir (10 mg) daily. Both tested negative for HBV-DNA by PCR (Amplicon HBV Monitor Assay, RT-PCR, Roche Molecular Systems, California) at 1 year after treatment with near normalization of ALT. Our plan is to withdraw manufactured by Orgentec Diagnostika GmbH, Germany. We then decided to look for the relatively uncommon causes of cirrhosis. On further investigation, his serum ceruloplasmin was 399 U/L, urinary copper<0.03 mg/L and KF ring was absent on slit lamp examination. Anti-nuclear, anti-mitochondrial and anti-smooth muscle antibodies tests were negative. Serum iron was 171 µg/dl, serum iron binding capacity was 305 µg/dl, serum ferritin was 205 ng/ml and serum α-1 anti-trypsin was 2.5 gm/L. In our quest for establishing the exact etiology of cirrhosis, especially considering the young age of the patient, we did HBV-DNA by PCR (Amplicon HBV Monitor Assay, RT-PCR, Roche Molecular Systems, California), which was positive with viral DNA load being 8×10⁷ copies/ml.

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antiviral if they remain undetectable for HBV-DNA by PCR on at least two more occasions at 6 months apart.

**Discussion**

The most important target for HBsAg serological assays is "a" determinant, which is located at the surface region of HBV spanning between amino acid 120-147. Mutations at "a" determinant result in reduced binding of anti-HBs and allow HBV to "escape" HBsAg immunoassay detection (19). HBV "a" escape mutants have been reported in apparently healthy individuals who are anti-HBc positive (20, 21), in children who have undergone anti-HBs seroconversion (22) and in HBsAg-negative chronic hepatitis patients (9, 23). It is yet to be known whether these mutants represent an escape mechanism for viral persistence and cause ongoing liver damage or not.

In seropositive occult HBV infection, mutations of S gene as well as in preS and S sequences have been reported (24). Mutations in S and X regions are known to affect HBV-DNA polymerase region due to overlapping open reading frames (ORFs) in the HBV genome. This may well be responsible also for occult HBV infection, because this inhibits the replicative efficacy of HBV thus leading to formation of low levels of HBsAg (24). In India, complete genomic analysis of 9 occult HBV infections revealed deletions at the junction of preS1 and preS2 as well as mutations in other regions. Liver biopsy was performed in 3 of these 9 patients and immunohistochemistry revealed abundant HBsAg in hepatocytes suggesting that HBsAg-negative occult HBV infection may be due to secretory defect of HBsAg from endoplasmic reticulum (25).

A Japanese study on 233 non-B non-C HCC patients detected HBV-DNA by PCR in 18% of them. All these patients were negative for HBsAg and HBcAg. The study established a link between occult HBV infection and its immense clinical importance (26). It has recently suggested that reactivation of occult HBV infection is an important cause of acute on chronic liver failure in the Asian Pacific region (27). Occult HBV infection can be categorized according to the presence of other serological markers of HBV. Seropositive occult HBV infection is those in which patients are anti-HBc and/or anti-HBs positive in addition to having detectable HBV-DNA by PCR, whereas those HBV infections where patients are only HBV-DNA positive by PCR, but negative for anti-HBc and anti-HBs are termed as seronegative occult HBV infection (24).

Several studies have reported that HBV-DNA can be detected by PCR in 55-78% of the patients up to 5 years after both spontaneous and/or treatment-induced loss of HBsAg (15, 21, 22). A study from India reported 9.5% occult HBV infection in a series of 591 patients with "cryptogenic" cirrhosis (25). Occult HBV infection is however rare in "healthy" individuals. For example young blood donors who are HBsAg-negative, but anti-HBs and/or anti-HBc-positive rarely test positive for HBV-DNA by PCR (10, 20, 28). However, significance of occult HBV infection and its long-term outcome remain unclear due to the small sample sizes in all the reported studies. However this is at least clear that in areas with high prevalence of HBV, diagnosis of "cryptogenic" cirrhosis based on HBsAg testing alone is not adequate.

Occult HBV infection is significant in a number of ways. Firstly, in HBV endemic areas, screening for HBV infection among cirrhotics using HBsAg alone is inadequate. Detection of HBV-DNA by PCR is warranted in HBsAg-negative patients, in whom no other etiology for cirrhosis can be detected. Secondly, the so called "cryptogenic" but actually occult HBV cirrhotics are suitable candidates for anti-viral treatment which would possibly slow down the progression of their liver disease. Thirdly, many of them require liver transplantation at some stage of their life. Identification of occult HBV infection is essential, as prophylactic anti-viral therapy must be offered to prevent re-infection of the transplanted liver by HBV. Fourthly, HBV-related liver cirrhosis carries high risk of development of HCC due to the integration of viral genome into host hepatocytes in addition to cirrhosis, which on its own is a pre-cancerous condition. This is also likely to be true in those with occult HBV related cirrhosis of liver. Finally, patients with occult HBV infection may transmit the infection to others through blood or organ donation.

**Conclusions**

Occult HBV infection must be considered in all patients with cryptogenic cirrhosis of liver in areas where HBV infection is prevalent. HBsAg alone is not sufficient to diagnose HBV infection and HBV-DNA assay by PCR is necessary before cirrhosis can be labeled "cryptogenic".

**References**