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Occult hepatitis B virus among chronic liver disease patients in Yemen

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ABSTRACT

Objective: To estimate the rate of occult hepatitis B virus (HBV) among patients with chronic liver disease (CLD). **Methods:** After an informed consent, sera samples were collected during April 2004 to April 2005 from 280 patients (200 male and 80 female). They were previously diagnosed with CLD based on history and ultrasound and were investigated for occult HBV infection. Sera were first screened for HBsAg and those which showed negative were tested for anti-HBc. The anti-HBc positive sera were further tested for anti-HBs to identify sera with isolated anti-HBc which in turn were subjected to HBV-DNA testing using PCR to determine the rate of occult HBV infection. Moreover, sera with occult HBV were tested for Anti-HCV and HCV-RNA using RT-PCR. **Results:** HBsAg was detected in 44 of 280 (15.7%). Of 236 HBsAg negative sera anti-HBc was detected in 22 (9.3%). All anti-HBc positive sera were found to be anti-HBs negative. HBV-DNA was detected in 11 of 22 (50.0%) sera with isolated anti-HBc indicating occult HBV in 4.3% of all sera. None of the sera with occult HBV had anti-HCV or HCV-RNA. **Conclusions:** Occult HBV infection does exist among CLD patients in Yemen and the mechanism of its occurrence merits further investigation.

1. Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the main viruses responsible for chronic viral hepatitis with its serious sequelae. An estimate of 2 billion people have been infected with HBV with more than 350 million are chronically infected worldwide^[1] and 170 million people worldwide, about 3% of the world population, are infected with HCV^[2]. The rates of chronic HBV carriers vary from 5 to 10%, among infected adolescents or adults, to upto 90% among neonates^[3] and the virus has been reported to be responsible for 80% of cirrhosis of the liver cases^[4].

Occult HBV is known as the presence of HBV viraemia without HBsAg in presence or absence of HBV antibodies outside the window period of the acute phase^[5]. Unlike conventional chronic HBV infection identified by serological markers occult HBV infection can only be identified by a molecular assay. The high sensitivity of PCR has facilitated

the detection of low level of HBV genome^[6,7]. The leading cause of occult HBV infection might be due to a mutant HBV that cannot be detected by HBsAg assays or more frequently by a virus that has suffered a strong suppression of viral replication and gene expression^[8,9]. Occult HBV has implications on transmission through blood transfusion, reactivation among immunosuppressed individuals and interference with hepatitis C treatment^[9]. It has been reported to have a role in a number of clinical phenomena including cryptogenic liver disease^[10], poor response to antiviral treatment^[11] and development of hepatocellular carcinoma in chronic HCV infected patients^[12]. The factors that play significant roles in occurrence of occult HBV infection includes the host's immune response, epigenetic factors and co-infection with HCV frequently reported with occult HBV^[13,14].

In this study we report the rates of occult hepatitis B among CLD patients in Yemen.

2. Materials and methods

2.1. Samples

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Table 1

Primers used for amplification of core and surface region of HBV-DNA and 5' end non-coding region of HCV-RNA (Invitrogen, life technologies USA).

DNA Detected	Primer sequence	Primer description
HBV	(5'to3') CGA CGT TGT AAA ACG ACG GCC AGT AAT GTC AAC AAT GTC AAC GAC CGA CGT TC	Outer sense of core region
HBV	(5'to3') CAG GAA ACA GCT ATG ACT AAC ACA GAA GCT CCA AAT TC	Outer anti-sense of core region
HBV	(5'to3') ATA CCA CAG AGT CTA GAC TC'	Outer sense of surface region
HBV	(5'to3') AAC TGA AAG CCA AAC AGT GG	Outer anti-sense of surface region
HBV	(5'to3') CAT AAG AGG ACTCTTGGAC	Inner anti-sense of core region
HBV	(5'to3') AAA GAA G/TTCAGAAGGCCAAAAAC/AGA	Inner-anti-sense of core region
HBV	(5'to3') CGA CGA TGT AAA ACG ACG ACG GCC AGT CTA GAC TCG TGG TGG ACT	Inner of surface region
HBV	(5'to3') CAG GAA ACA GCT ATG ACC TAC GAA CCA CTG AAC AAA TGG CAC	Inner-anti-sense of surface region
HCV	(5'to3') CTGTGAGGAAGTACTACTGTCTT	Inner sense of 5' end non-coding region
HCV	(5'to3') ATACTCGAGGTGCACGGTCTACGAGACCTT	Inner-anti-sense of 5' end non-coding region
HCV	(5'to3') TCTGTGTGTAAGACTCACC	Outer sense of 5' end non-coding region
HCV	(5'to3') TCACTCTCGAGCACCTATCAGGCAGT	Outer anti-sense of 5' end non-coding region

Anonymised frozen (-20 °C) sera which were collected during the period from April 2004 to April 2005 from 280 patients who were diagnosed with CLD based on history and ultrasound was tested for HBsAg. Sera which were negative for HBsAg were tested for anti-HBc and those which showed positive anti-HBc were further tested for anti-HBs to identify sera with isolated anti-HBc. Sera with isolated anti-HBc were subjected to testing for HBV-DNA to determine the rate of occult HBV. All HBV-DNA positive sera were analysed for anti-HCV and further confirmed through HCV-RNA detection.

2.2. Serological investigations

Sera were tested for HBsAg, anti-HBc and anti-HCV using microparticle enzyme immunossay (MEIA)/ IMx system (Abbott, USA).

2.3. Nucleic acid detection

Nucleic acid extraction and amplification by two sets of primers that targets the core and surface region of the HBV has been previously described^[15]. Primers which were used are described in Table 1. Reverse transcription of HCV-RNA was performed in a final volume of 20 µL using 10.7 µL of extracted RNA with 2.5 µM random hexamer (Amersham Pharmacia Biothec, Inc, USA), 100 units of Moloney Murine reverse transcriptase (Invitrogen, UK) and 200 µM dNTPs. The reaction conditions were 42 °C for 60 minutes, 50 mM tris HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂. In a total volume of 25 µL the amplification was performed on 10 µL of cDNA using 200 µM dNTPs (Helena Biosciences, UK) and 0.63 units of taq polymerase (Invitrogen, UK). The reaction conditions of the first PCR round were 20 mM tris HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 40 cycles consisted denaturation of 94 °C for 1 minute, annealing of 45 °C for 1 minute and extension of 72 °C for 1 minute. The second round PCR was performed on 1 µL of the first round product under the same conditions of the first round. Amplification of DNA was checked by gel electrophoresis of 10 µL of the second stage PCR product at 100 volts for 1 to 1.5 % using agarose gel.

3. Results

HBsAg was detected in 44 of 280 (15.7%). Among the 236 HBsAg negative sera anti-HBc was detected in 22 of 236 (9.3%). All the anti-HBc positive sera were anti-HBs negative. HBV-DNA was detected in 11 of 22 (50.0%). Sera with isolated anti-HBc indicating occult HBV infection among 4.3% of all patients (Median age 40, ranging from 22 to 54). None of the patients with occult HBV infection had anti-HCV or HCV-RNA.

4. Discussion

A total of 4.3% of CLD patients had occult HBV infection as indicated by the detection of HBV-DNA in their sera (this is the only marker in addition to anti-HBc) using PCR assay with sensitivity of 100 copies/mL^[15]. This has not been previously reported from Yemen. The absence of HBsAg in these patients is unlikely to be ascribed to false negative test results but due to very low HBsAg concentrations, as the assay used has a "reported" sensitivity of 97.7%. Concomitant HCV infection has been well documented to down regulate HBV replication and to suppress expression of HBsAg^[16-18]. None of the patients had an HCV co-infection by a serological assay and RT-PCR. However, it is unknown whether these patients had previously recovered from HCV infection (as a result of influence of HBV co-infection). Early study by Chu *et al* reported that the persistence of HCV and antibody response to it was found to be inhibited by active replication of HBV^[19]. In addition to that occult HBV infection has been reported to associate with HIV^[20-22]. However, absence of data on the status of HCV-RNA and HIV antibodies limited drawing a conclusion on the role of the HCV and HIV co-infection in development of occult hepatitis among our patients. The origin of occult HBV has been hypothesized to be due to mutations in the regulatory regions of the HBV genome^[23]. A novel splicing event of HBV-RNA has been suggested to abolishes surface protein

expression without affecting other functions encoded in the virus genome P, C and X^[24]. Whether or not this had happened in our patients is not clear. However these patients might be at risk of reinfection and might become candidates for orthotropic liver transplants. Moreover, immunosuppressive treatment may also put this patient at risk of reactivation. As occult hepatitis B is increasingly reported among patients with CLD^[24–29] awareness of this condition among clinician should be increased with an ultimate aim of proper management of such patients. Consequently, how relevant the CLD among our patient is to their occult HBV infection merits further investigation.

Conflict of interest statement

We declare that we have no conflict of interest.

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