

Absence of hepatitis B virus DNA in patients with hepatitis C and non-A-E hepatitis in the State of São Paulo, Brazil

L.O. Souza^{1,2},
J.R.R. Pinho^{1,4},
F.J. Carrilho³ and
L.C. da Silva³

¹Serviço de Virologia, Instituto Adolfo Lutz, São Paulo, SP, Brasil
²Departamento de Microbiologia, Instituto de Ciências Biomédicas, and
³Departamento de Gastroenterologia, Faculdade de Medicina,
Universidade de São Paulo, São Paulo, SP, Brasil
⁴Laboratório Bioquímico Jardim Paulista, São Paulo, SP, Brasil

Abstract

Occult hepatitis B virus (HBV) infection has been reported as cases in which HBV DNA was detected despite the absence of any HBV serological markers or in cases in which anti-HBc antibody was the sole marker. The aim of the present study was to determine, using the polymerase chain reaction (PCR), whether HBV infection occurs in hepatitis C and non-A-E hepatitis patients without serological evidence of hepatitis B infection in São Paulo State. Two different populations were analyzed: 1) non-A-E hepatitis patients, including 12 patients with acute and 50 patients with chronic hepatic disorders without serological evidence of infection with known hepatitis viruses; 2) 43 patients previously diagnosed as hepatitis C with positive results for anti-HCV and HCV RNA. Among hepatitis C patients, anti-HBc was detected in 18.6% of the subjects. Three different sets of primers were employed for HBV DNA detection by nested PCR, covering different HBV genes: C, S and X. HBV-DNA was not detected in any sample, whereas the positive controls did produce signals. The lack of HBV DNA detection with these pairs of primers could be due to a very low viral load or to the presence of mutations in their annealing sites. The latter is unlikely as these primers were screened against an extensive dataset of HBV sequences. The development of more sensitive methods, such as real time PCR, to detect circular covalent closed DNA is necessary in order to evaluate this question since previous studies have shown that cryptic hepatitis B might occur.

Key words

- Hepatitis B virus
- Non-A-E hepatitis
- Polymerase chain reaction

Correspondence

J.R.R. Pinho
Serviço de Virologia
Instituto Adolfo Lutz
Av. Dr. Arnaldo, 355
01246-902 São Paulo, SP
Brasil
Fax: +55-11-3088-3753
E-mail: jrpinho@usp.br

Research supported by FAPESP
(Nos. 97/04955-0 and 99/09745-0).

Received September 22, 2003
Accepted June 28, 2004

Cases of occult hepatitis B virus (HBV) infection have been reported, representing those in which HBV DNA was detected despite the absence of any serological HBV markers, or those on which the anti-HBc

antibody was the sole marker. Bréchet et al. (1) studied patients with chronic liver disease and detected HBV-DNA sequences in 52 (59%) of 88 liver samples and in 10 (9.5%) of 105 serum samples (6 of which

had no serologic HBV marker). Paterlini et al. (2) detected HBV DNA in 6 of 13 HBsAg-seronegative patients with hepatocellular carcinoma (HCC). All of these patients were also HBcAg negative in liver tissue. Using polymerase chain reaction (PCR), Uchida et al. (3) amplified HBV DNA in 18 (90%) cases of acute hepatitis and 17 (85%) cases of chronic hepatitis among patients serologically diagnosed with non-A-C hepatitis. These patients were infected with viruses showing mutations in the HBV X gene, which were tentatively called hepatitis F.

Several investigators have also reported silent HBV co-infection in hepatitis C patients. Ruiz et al. (4) found this co-infection in 9 (14%) of 63 patients with HCC, all of them being HBsAg negative. Paterlini et al. (5) detected HBV infection in 6 cases of HCC, all of them seronegative for hepatitis B markers, including 2 patients simultaneously positive for hepatitis C virus (HCV) RNA. Feitelson et al. (6) observed this co-infection in HBV-seronegative β -thalassemic children previously vaccinated against hepatitis B. The HBV isolates from these cases showed deletions in the X gene similar to those found by Uchida et al. (3) and Feitelson et al. (7). Cacciola et al. (8) detected HBV-DNA in one third of HBsAg-seronegative patients with chronic hepatitis C and suggested an association between cirrhosis in HCV-positive patients with the presence of occult HBV infection. Fukuda et al. (9) found hepatitis B infection in 22 (48%) of 45 patients with hepatitis C and related it to a low response to interferon treatment and to higher levels of HCV RNA.

The aim of the present study was to determine, by PCR, whether occult HBV DNA occurs in patients with hepatitis C and non-A-E hepatitis in São Paulo State.

The Ethics Committee of the Instituto de Ciências Biomédicas, Universidade de São Paulo, approved this research and written informed consents were obtained from all patients.

Two populations were analyzed: 1) non-A-E hepatitis patients, including 12 patients with acute and 50 patients with chronic hepatic disorders without serological evidence of infection with known hepatitis viruses; 2) 43 patients previously diagnosed as hepatitis C with positive results for anti-HCV and HCV RNA. Serum samples collected from patients were kept at -20°C until the time for use. All of these patients had their serological results confirmed using commercially available kits to detect HBsAg, anti-HBs, anti-HBc (DiaSorin, Saluggia, Italy) and anti-HCV (Abbott Laboratories, North Chicago, IL, USA). All non-A-E hepatitis cases were also tested for anti-HEV (Abbott Laboratories). Acute non-A-E hepatitis cases were also tested for anti-HAV IgM, anti-HBc IgM (Abbott Laboratories) and HCV RNA by RT-PCR, after guanidine isothiocyanate extraction. Samples with positive results for HBsAg, anti-HBs or anti-HBc were also tested for HBeAg, anti-HBe and anti-HBc IgM (DiaSorin).

Three different sets of primers were employed for HBV DNA detection by nested PCR, covering different HBV genes: C (10), S (11) and X (3). Sample treatment before amplification and cycling were carried out as described by Kaneko et al. (10). Positive (positive HBV DNA samples from HBsAg-positive patients) and negative controls (HBV DNA-negative samples from patients with liver steatosis) were included in all reaction runs.

Among the non-A-E hepatitis patients, HBsAg, anti-HBc, anti-HCV, anti-HEV, and HCV RNA results were confirmed as negative. Anti-HAV IgM, anti-HBc IgM and HCV RNA were negative in the acute cases. Anti-HBs was the only positive marker in three patients, a pattern found in previously vaccinated individuals.

Among hepatitis C patients, anti-HBc was detected in 8 (18.6%) subjects, confirming that HBV infection is frequently found in this group. Of these 8 patients, 5 were also anti-HBs antibody positive, two were posi-

tive for anti-HBc IgM and 4 were anti-HBe antibody positive; only one patient was positive for anti-HBc antibody alone. Two patients presented anti-HBs as the sole marker, which is compatible with previous vaccination. However, no positive results were obtained with any PCR set. Perhaps, these patients had a viral load below the detection limit of nested PCR or perhaps the high sensitivity and/or lack of specificity of the anti-HBc diagnostic test produced false-positive results.

The absence of detectable HCV RNA in any sample, while the positive controls did produce signals, is strikingly different from previously reported studies (1,2,4,5,8).

It is worth noting that the primers used cover three different HBV genes. The sensitivity of the methodology developed by Kaneko et al. (10) was estimated as 3 copies per tested sample (i.e., 300 copies per ml). Using the same methodology, we were able to detect HBV DNA in more than 90% of chronic HBV cases (12). This procedure has also been used in another study involving patients who developed HBsAg clearance and allowed the early detection of HBV DNA in one patient who relapsed after clearance (13).

Primers directed at the S region were chosen after the finding of occult HBV infection by others (8,14). Although some of the primers covering this region overlap with those previously reported, we found no case which was HBV DNA positive.

Finally, we applied primers from the X region described by Uchida et al. (3) that are allegedly able to detect occult hepatitis B in case this region is mutated. Even using this third set of primers, no occult hepatitis B case was detected.

The lack of HBV DNA detection with these pairs of primers could be due to a very low viral load, below the reaction threshold, or to the presence of mutations in their annealing sites. The latter is, however, unlikely for these primers were screened against an

extensive dataset of HBV sequences and few mutations were observed in the annealing region. The use of other methodologies such as the detection of circular covalent closed HBV DNA in liver tissue by real time PCR would be helpful to clarify this matter (15).

It has been shown that viral DNA can be detected for a long time after the resolution of hepatitis (16), probably representing remnants of degraded liver cells. Therefore, new studies with real time PCR should be of help to evaluate the clinical meaning of the detection of very low levels of HBV DNA. In fact, Saito et al. (17) reported that silent hepatitis B patients usually have a very low viral load.

Sagnelli et al. (18) observed that isolated anti-HBc in hepatitis C patients is clinically relevant and this might be explained by the fact that HCV interferes with HBV replication. In such cases, low levels of HBV DNA would remain in the hepatocytes, explaining the severity of the disease and its association with HCC. Furthermore, we would like to emphasize that the presence of detectable anti-HBc IgM in two anti-HCV-positive patients was not associated with detectable HBV DNA. Other investigators have reported the finding of weakly positive anti-HBc IgM levels in chronic patients without current evidence of HBV replication (19).

Santos et al. (20) reported that, among patients infected with human immunodeficiency virus (HIV) from Rio de Janeiro, HBV DNA was detectable by PCR of the core and pre-S regions in 12/60 (20%) of the anti-HBs-positive samples and in 4/41 (10%) of the samples positive for anti-HBc only, respectively. A similar study is yet to be carried out on HIV-infected patients in São Paulo State.

HBV DNA could not be detected in HbsAg-negative cases, including non-A-E hepatitis and C cases. In view of previous findings that cryptic hepatitis B might occur in our country, the development of more sensitive methods that could detect circular covalent closed DNA, such as real time PCR, would greatly help.

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