۳۰ درصد تخفیف نوروزی ویژه کارگاه‌ها و فیلم‌های آموزشی

اصول تنظیم قراردادها
پروپوزال نویسی
آموزش مهارت های کاربردی در ندوین و چاب مقاومت

پیش
Occult Hepatitis B Virus Infection in Patients With Chronic Hepatitis C Treated With Antiviral Therapy

Gian Paolo Caviglia 1, Maria Lorena Abate 1, Paola Manzini 2, Franca Danielle 2, Alessia Ciancio 3, Chiara Rosso 1, Antonella Olivero 1, Rinaldo Pellicano 3*, Giovanni Antonio Touscoz 3, Antonina Smedile 3, Mario Rizzetto 3

1 Department of Internal Medicine, University of Turin, Turin, Italy
2 Blood Bank, San Giovanni Battista University Hospital (Molinette), Turin, Italy
3 Department of Gastroenterology and Hepatology, San Giovanni Battista University Hospital (Molinette), Turin, Italy

ARTICLE INFO

Article type: Original Article
Article history:
Received: 01 Jul 2012
Revised: 10 Jul 2012
Accepted: 28 Jul 2012

Keywords:
Hepatitis C, Chronic
Hepatitis B Virus
Hepatitis C Infection

ABSTRACT

Background: Occult hepatitis B virus infection (OBI) is defined as the presence of hepatitis B virus (HBV) DNA in the liver and/or in the serum of patients with negative results of hepatitis B s antigen (HBsAg) test with or without serological markers of previous viral exposure. The impact of OBI in patients with chronic hepatitis C (CHC) is still unclear.

Objectives: The Aim of this study was to assess OBI prevalence and its potential implications on treatment outcome in a cohort of patients with CHC underwent standard antiviral therapy.

Patients and Methods: Baseline serum samples from 137 HBsAg-negative CHC patients treated with pegylated-interferon and ribavirin (73 Responders/74 Non Responders), were retrospectively analyzed for HBV status.

Results: Seventy-three patients (53.3%) showed markers of previous exposure to HBV. HBV DNA was detected in 2 of 137 serum samples (1.5%), both carrying HBV antibodies. Liver biopsies and post-therapy sera were available for 35 patients (12 Responders/23 Non Responders). HBV DNA sequences were found in 13 of 35 specimens (37.1%), all of patients with HBV DNA negativity in basal and post-therapy serum samples. Among OBI-positive patients, 5 (38.5%) carried serological markers of HBV infection. Regarding therapy outcome, in the OBI-positive group there were 5 of 13 (38.5%) sustained virological responders (SVR) compared to 7 of 22 (31.8%) in the OBI-negative one.

Conclusions: Despite the high prevalence rate of liver HBV DNA in patients with CHC, SVR was not affected by occult HBV infection.

Implication for health policy/practice/research/medical education: This article contributes to enrich the panorama of the management of patients with chronic HCV hepatitis and occult HBV infection.

Please cite this paper as:

* Corresponding author: Rinaldo Pellicano, Department of Gastroenterology and Hepatology, San Giovanni Battista University Hospital, C.so Bramante n.88/90, 10100 Turin, Italy. Tel: +39 0116333532, Fax: +39 0116333976, E-mail: rinaldo_pellican@hotmail.com

DOI: 10.5812/hepatmon.7292
© 2012 Baqiyatallah Research Center for Gastroenterology and liver diseases; Published by Kowsar Corp. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
1. Background

Occult hepatitis B virus infection (OBI) is defined as the presence of hepatitis B virus (HBV) DNA in the liver of patients with negative results of hepatitis B s antigen (HBsAg) test with or without serological markers of previous viral exposure (1-3). In these patients, the lack of circulating HBsAg may be due to rearrangements in the HBV genome which interfere with gene expression or lead to the production of an antigenically modified S protein (4-6). The molecular basis of occult HBV infection is related to the long-lasting persistence in the nuclei of hepatocytes of the viral covalently-closed-circular DNA (cccDNA) (7). Almost all OBI cases are infected with replication-competent HBV showing strong suppression of replication and gene expression, probably due to host immune-surveillance and epigenetic factors (8). On the basis of HBV antibodies profile, OBI may be distinguished in seropositive- and seronegative-OBI; the former is positive for hepatitis B core and/or S antibodies, the latter is negative for all markers of HBV infection aside very low amount of HBV DNA (< 200 IU/ml) (8). Seronegative-OBI cases may have either progressively lost HBV specific antibodies after the resolution of an acute infection or, theoretically, had negative results of tests from the beginning of infection, similar to what has been observed in the woodchuck model of hepadnavirus infection with the woodchuck hepatitis virus (9), where a low-dose infection was insufficient to allow the maturation of an antiviral protective memory response (10). Although OBI status is significantly associated with the presence of antibodies to HBV (3), the analysis of liver DNA extracts represents the gold standard for occult HBV evaluation (8). Hence, serum analysis must be taken into account only in the absence of liver specimens. In any case, it is strongly recommended to use a highly sensitive nested polymerase chain reaction (PCR) or real time PCR with oligonucleotide primers specific for different HBV genomic regions and complementary to highly conserved nucleotide sequences (11). Occult HBV infection has been found with a high prevalence in patients with chronic hepatitis C (CHC), probably because both HBV and hepatitis C virus (HCV) share the same parenteral way of transmission. In particular, HBV DNA is detectable in about one-third of CHC patients with negative results of HBsAg test in the Mediterranean basin (3).

2. Objectives

Since it is unclear the impact of OBI in patients with CHC, the aim of this study was to evaluate OBI prevalence and its possible implications on antiviral therapy outcome in a cohort of patients treated for CHC.

3. Patients and Methods

One hundred thirty seven CHC patients with negative results of HBsAg test, treated with pegylated interferon (peg-IFN) and ribavirin, were included in the study. The study was performed according to the 1975 Declaration of Helsinki. Written informed consent was obtained from all patients prior to recruitment. The main demographic characteristics are reported in Table 1. Baseline serum samples were analyzed for HBV antibodies (anti-HBs, anti-HBc and anti-HBe) with standard assays. For HBV DNA detection, in baseline and selected six month follow-up serum samples, viral nucleic acids were purified from a 400 µl specimen by automated procedure (EZI Virus Mini Kit v.2, BioRobot EZI Advanced, QIAGEN). Liver biopsies were available for a subgroup of 35 serum matched patients. Frozen liver specimens (10-12 mg) were disrupted in TRIZOL by a rotor-stator homogenizer. After purification, liver DNA concentration and quality were assessed with spectrophotometer (NanoDrop ND 1000, NanoDrop Technologies). All serum and liver nucleic acids extracted were tested for HBV DNA by four parallel nested-PCRs to detect HBV S, Core, and Pol and X sequences (detection limit 5 IU/ml). PCR primers were complementary to highly conserved nucleotide sequences of HBV genome (8). Two rounds of amplification, 35 cycles each, were performed using HotStartTaq Polymerase (Qiagen, Germany). Appropriate negative and positive controls were included in each PCR experiment. To check for false negatives a parallel PCR for beta-globin gene was performed. In addition, direct sequencing of amplicons obtained by nested PCR was performed to confirm the specificity of the reactions. Samples turned positive for at least two gene targets were scored as OBI-positive according to Taormina expert meeting statements (8). Statistical analysis was performed using Student’s t test or Mann-Whitney U test to compare continuous variables and Fisher’s exact test to analyze categorical data. All P values are two-tailed; a P value below 0.05 was considered statistically significant.

4. Results

Among the 137 HCV patients with negative results of HBsAg test, HBV antibodies were detected in 73 (53.3%) (Table 2), while serum HBV DNA was detected only in 2 (1.5%), both positive for the four HBV genomic regions were examined (S, Core, Pol, X). Each of them carried markers of previous HBV exposure (one positive for anti-HBs, anti-HBc and anti-HBe, the other positive for both anti-HBc and anti-HBe). One patient was responder to HCV therapy while the other was not. Because of low number of cases with positive results of HBV DNA test in their sera, no statistical correlation was detected. Liver biopsies were available for 35 of 137 patients. Intra-hepatic HBV DNA was detected in 13 of 35 patients (37.1%) with nested PCR for at least two different HBV genomic regions, but none of the corresponding basal or follow-up serum samples tested positive. In detail, two cases had positive results of all four HBV regions examined; six had positive results for three regions (S, Core and Pol) and 5 for two regions (three for S and Core; one for Core and Pol; one for S and Pol). Eight of...
13 patients with occult HBV infection had negative results for serum anti-HBV antibodies test, whereas the remaining five were seropositive for at least one marker of previous HBV exposure (one for anti-HBc, two for anti-HBc and anti-HBs, one for anti-HBc and anti-HBe, one for anti-HBs). Their characteristics are reported in Table 3. There was no statistical difference between OBI-positive and OBI-negative patients regarding sex, age, HBV antibodies, aminotransferase levels, liver fibrosis and basal HCV viral load. Patients who responded to HCV antiviral treatment were equally distributed between HBV DNA-positive and -negative patients: 5/13 (38.5%) versus 7/22 (31.8%; P = 0.726), respectively.

5. Discussion

In the present study serum HBV DNA was detected in two of 137 (1.5%) CHC patients with negative results of HBsAg test. A higher prevalence rate was found in liver, where HBV sequences were detected in 13 of 35 (37.1%) specimens. Among the 13 OBI-positive patients, only five (38.5%) carried serological markers of HBV infection. The prevalence rate of OBI in patients with CHC patients reported in the literature varies greatly, ranging from 0% to 52% (12, 13). This wide range might be linked to the geo-

---

**Table 1. Baseline Characteristics of Patients**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All Patients</th>
<th>Liver Biopsy Subgroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>137</td>
<td>35</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>89/48</td>
<td>22/13</td>
</tr>
<tr>
<td>Age, y, range</td>
<td>48.0 (29-69)</td>
<td>48.4 (29-68)</td>
</tr>
<tr>
<td>HCV genotype 1, No. (%)</td>
<td>78 (56.9%)</td>
<td>28 (80%)</td>
</tr>
<tr>
<td>Therapy outcome R vs. NR</td>
<td>73 vs. 64</td>
<td>12 vs. 23</td>
</tr>
</tbody>
</table>

Abbreviations: M, male; F, female; HCV, hepatitis C virus; R, responders; NR, non-responders.

**Table 2. Serum Markers of Previous HBV Exposure**

<table>
<thead>
<tr>
<th>HBV markers positive, No. (%)</th>
<th>All Patients</th>
<th>Liver Biopsy Subgroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HBc positive</td>
<td>73 (53.3%)</td>
<td>19 (54.3%)</td>
</tr>
<tr>
<td>Anti-HBc/anti-HBs positive</td>
<td>24 (17.5%)</td>
<td>7 (20%)</td>
</tr>
<tr>
<td>Anti-HBc/anti-HBe positive</td>
<td>9 (6.6%)</td>
<td>2 (5.7%)</td>
</tr>
<tr>
<td>Anti-HBc/anti-HBs/anti-HBe positive</td>
<td>13 (9.5%)</td>
<td>1 (2.9%)</td>
</tr>
<tr>
<td>Anti-HBs positive</td>
<td>2 (1.5%)</td>
<td>2 (5.7%)</td>
</tr>
</tbody>
</table>

Abbreviations: HBV, hepatitis B virus; anti-HBc, antibodies to hepatitis B core antigen; anti-HBs, antibodies to hepatitis B surface antigen; anti-HBe, antibodies to hepatitis B e antigen.

**Table 3. Characteristics of Patients With Liver Biopsy (n = 35) According to OBI Status**

<table>
<thead>
<tr>
<th></th>
<th>OBI positive</th>
<th>OBI negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>13 (37.1%)</td>
<td>22 (62.9%)</td>
<td></td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>10/3</td>
<td>12/10</td>
<td>0.282</td>
</tr>
<tr>
<td>Age, y, range</td>
<td>50.1 (31-68)</td>
<td>47.4 (29-66)</td>
<td>0.681</td>
</tr>
<tr>
<td>AST, IU/L, mean ± SD</td>
<td>82 ± 35</td>
<td>63 ± 32</td>
<td>0.112</td>
</tr>
<tr>
<td>ALT, IU/L, mean ± SD</td>
<td>136 ± 84</td>
<td>102 ± 64</td>
<td>0.183</td>
</tr>
<tr>
<td>Ishak histology fibrosis score, mean ± SD</td>
<td>2.69 ± 0.63</td>
<td>2.86 ±1.46</td>
<td>0.863</td>
</tr>
<tr>
<td>Ishak histology activity score, mean ± SD</td>
<td>4.69 ±1.25</td>
<td>4.45 ± 2.22</td>
<td>0.483</td>
</tr>
<tr>
<td>HCV genotype 1, No. (%)</td>
<td>11 (85%)</td>
<td>17 (77%)</td>
<td>0.689</td>
</tr>
<tr>
<td>Basal HCV Viral load, IU/ml, median</td>
<td>2.0 × 106</td>
<td>1.6 × 106</td>
<td>0.441</td>
</tr>
<tr>
<td>Therapy outcome R vs. NR</td>
<td>5 vs. 8 (38.5%)</td>
<td>7 vs. 15 (31.8%)</td>
<td>0.726</td>
</tr>
<tr>
<td>HBV markers positive, No. (%)</td>
<td>5 (38.5%)</td>
<td>14 (63.6%)</td>
<td>0.179</td>
</tr>
<tr>
<td>Anti-HBc positive</td>
<td>1 (7.7%)</td>
<td>6 (27.3%)</td>
<td></td>
</tr>
<tr>
<td>Anti-HBc/anti-HBs positive</td>
<td>2 (15.4%)</td>
<td>5 (22.7%)</td>
<td></td>
</tr>
<tr>
<td>Anti-HBc/anti-HBe positive</td>
<td>1 (7.7%)</td>
<td>1 (4.6%)</td>
<td></td>
</tr>
<tr>
<td>Anti-HBc/anti-HBs/anti-HBe positive</td>
<td>0</td>
<td>1 (4.6%)</td>
<td></td>
</tr>
<tr>
<td>Anti-HBs positive</td>
<td>1 (7.7%)</td>
<td>1 (4.6%)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: OBI, occult hepatitis B virus infection; M, male; F, female; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus; R, responders; NR, non-responders; HBV, hepatitis B virus; anti-HBc, antibodies to hepatitis B core antigen; anti-HBs, antibodies to hepatitis B surface antigen; anti-HBe, antibodies to hepatitis B e antigen.
graphical distribution of HBV infection (14) as well as to
the sensitivity of the method used to detect HBV DNA, in-
cluding PCR primer selection (15), and OBI definition (13).
Examination of liver DNA extracts is the “gold standard”
for OBI testing, but it is not always applicable in clinical
practice (2, 13). In fact, HBV DNA is detected in liver tissue
specimens of patients with serum HBV DNA but is often
undetectable in serum of patients with intra-hepatic
HBV DNA (16). For this reason, if a liver biopsy specimen
is not available, analysis of serum samples should be
performed with a highly sensitive and specific approach
based on nested PCR or real time PCR. Moreover, it is re-
commended to perform serum HBV detection at differ-
tent time points because the analysis of only one sample,
drawn before therapy, could not be sufficient to detect
OBI if virus replication is intermittent (17). Many studies
reported a higher prevalence of HBV-DNA detection in
anti-HBc-positive than in anti-HBc-negative patients (3,18,
19). In our cohort, similarly to what reported by several
authors (10, 15, 20, 21), since 8 of 13 patients with positive
results of OBI test were seronegative, OBI was not associ-
ated with the presence of anti-HBc antibodies. Previous
reports (12, 15) showed that serum HCV-RNA load was
significantly higher in CHC OBI-positive patients than
in negative ones. In our study, in agreement with other
groups (22-25), no statistically significant differences
were found. To evaluate whether occult HBV infection
contributes to liver damage in CHC patients, we com-
pared histological scores according to the presence of
HBV DNA. As previously reported by other authors (20,
24, 25) we did not find any association between OBI and
severity of chronic liver disease. However, other studies
(2,15,16) found that severe lesions were more common in
CHC patients with positive results of OBI test compared
to those with HCV infection alone. Chen et al. (23) report-
ed that patients with both OBI and HCV infection had
lower ALT levels, liver histology activity index and fibrosis
scores than those with HCV monoinfection. The clinical
impact of OBI on anti-HCV therapy outcome is still con-
troversial. Preliminary studies, focusing on the treatment
response to IFN monotherapy, suggested an association
between the presence of OBI and a lower virological re-
response rate (12, 16). Although some recent studies (20, 24,
26) with peg-IFN plus ribavirin therapy, showed that the
presence of OBI had no or minimal effect on the outcome,
while others did not confirm these data (15). In the pres-
ent study, patients responders to HCV antiviral treatment
were equally distributed between patients with positive
and negative results of HBV DNA testing (38.5% versus
31.8%, respectively) confirming that the rate of sustained
virological response to peg-IFN plus ribavirin combina-
tion therapy is similar in the two groups. Finally, a recent
study (27) showed a strong association between the pres-
ence of OBI in patients with CHC and the development of
hepatocellular carcinoma (HCC) when compared to pa-
tients with monoinfected HCV. Moreover, OBI was strong-
ly associated with liver cancer independently of age, sex,
HCV co-infection and cirrhosis. These findings suggested
that OBI might contribute to hepatocyte transformation,
playing a direct oncogenic role through both its integra-
tion into the host genome and a maintained transcrip-
tional activity, allowing the synthesis of proteins with
potential pro-oncogenic properties. In the present study,
we was not possible to draw such conclusion because none
of the 35 patients developed HCC in the two years after
the end of treatment. Nevertheless, a closer follow-up is
recommended in patients with positive results of OBI
test for the potential risk of HCC development.

Acknowledgments

None declared.

Authors’ Contribution

Gian Paolo Caviglia and Maria Lorena Abate: Co-authors
of study design collected and analyzed the data, wrote
the manuscript. Rinaldo Pellicano: Critical revision of
the manuscript for important intellectual content. Paola
Manzini, Franca Daniele, Alessia Ciancio, Chiara Rosso,
Antonella Olivero, and Giovanni Antonio Touscoz: Manu-
script writing contributed new reagents or analytic tools
and performed research. Antonina Smedile: Design of
the study, analysis and interpretation of the data, critical
revision. Mario Rizzetto: Guarantor of integrity of the en-
tire study.

Financial Disclosure

None declared.

Funding/Support

None declared.

References

1. Brechot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Pa
erlini-Brechot P. Persistent hepatitis B virus infection in subjects with-
out hepatitis B surface antigen: clinically significant or purely “occult”? Hepatol
2. Raimondo G, Pellicano T, Cacciola L, Squadrito G. Occult hepatitis B
2002;2(8):479-86.
occurring missense mutation in the polymerase gene terminat-
5. Carman WF, Van Deursen FJ, Mimms LT, Hardie D, Coppola R,
Decker R, et al. The prevalence of surface antigen variants of hep-
atitis B virus in Papua New Guinea, South Africa, and Sardinia.
S, et al. Naturally occurring escape mutants of hepatitis B virus
with various mutations in the S gene in carriers seropositive for
7. Levreco M, Pellicino T, Petersen J, Belloni L, Raimondo G, Dandri
M. Control of cccDNA function in hepatitis B virus infection.
8. Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, Co-
Caviglia GP et al. OBI and Chronic Hepatitis C Treatment


30 درصد تخفیف نوروزی ویژه کارگاه‌ها و فیلم‌های آموزشی

اصول تنظیم قراردادها

بروپوزال نویسی

آموزش مهارت‌های کاربردی در تدوین و جاب مقاله