Occult Hepatitis B virus Infection in Egyptian Patients with Hepatitis C Virus Related Chronic Liver Disease

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Concurrent infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) are increasingly recognized in patients with both acute and chronic hepatitis and the reciprocal influence of dual infection remains controversial. In Egypt, the last decade showed a remarkable decline in HBV infection associated with remarkable rise in HCV infection. This study investigates the prevalence of occult HBV in adults with HCV related chronic liver disease (CLD) to spotlight its importance on the clinical outcomes.

Ninety five patients with HCV related chronic liver disease (median age 50 yrs) were enrolled in this study. Thirty of them were suffering from hepatocellular carcinoma (HCC). Sera were tested for HCV antibodies, HCV-RNA (nested RT-PCR), HBV markers (HBsAg, Anti-HBcAb IgM & total, HBeAg) and HBV-DNA (nested PCR for s, c & x regions).

All the studied patients were anti-HCV positive, where 47/95 (49.5%) of them were HCV RNA positive. HBsAg was detected in 25/95 (26.3%) (Overt HBV infection), Total anti-HBc was detected in 52/83 (62.6%), HBV-DNA was positive among 41/95 (43.1%) with greatest prevalence for "c" region 39/95 (41%). H BV DNA positive / HBsAg negative (occult HBV infection) was significantly prevalent in HCV-CLD vs HCC patients (p<0.001), and was found to be significantly increased in those who were HCV RNA positive rather than in HCV RNA negative patients (P< 0.05). No significant difference was detected between patients with occult or overt HBV infection as regard to liver enzymes or Child classification (P>0.05).

Occult HBV infection was found to be significantly increased in HCV related chronic liver disease with (p<0.05). The high prevalence occult HBV-infection (particularly core DNA) may have clinical implications in the pathogenesis and therapy of HCV induced chronic liver disease. Standardized definition and diagnostic criterion of occult HBV infection are needed for future research to determine its prevalence and clinical significance.

INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infection account for a substantial proportion of chronic liver disease (CLD) including chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). It is estimated that there are 350 million HBV carriers and 170 million HCV carriers worldwide. Both HBV and HCV have the same routes of transmission, thus concurrent infections with both viruses are increasing in patients with acute and chronic hepatitis particularly in areas where the two viruses are endemic and among people at high risk for parental infections.

The interaction between HCV and HBV has so far been poorly investigated, little is known about the clinical presentation, the natural history and the response to antiviral treatment of liver diseases associated with HBV and HCV coinfection.

The diagnosis of HBV infection is usually based on the detection of hepatitis B surface antigen (HBsAg) and the disappearance of this antigen indicates the clearance of HBV. However, 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, or even after a successful anti-HBV treatment. Demonstration of this clinical entity has resulted in introduction of the concept of occult HBV infection, which is defined as the detection of HBV-DNA without HBsAg with or without the presence of HBV antibodies outside the acute phase window period. Occult HBV infection has frequently been identified in patients with chronic HCV infection and in such patients this occult infection may be associated with more severe liver damage and even the development of hepatocellular carcinoma (HCC). Despite its potential clinical importance, the prevalence of occult HBV infection in patients with HCV is still undetermined. Rodriguez-Inigo et al. stated that most patients who have dual HBV
and HCV infections have detectable serum HCV-RNA but undetectable or low HBV-DNA level, indicating that HCV is the predominant cause of liver disease in these patients.

The aim of this work is to analyze the prevalence of occult HBV infection in Egyptian patients with HCV related chronic liver diseases.

PATIENTS & METHODS

Study Design:
This is a cross-sectional retrospective study that included ninety five patients with chronic liver disease (50 males, 45 females). They were consecutively enrolled from outpatients and inpatients regularly attending The Tropical and Internal Medicine Departments at Al-Zahrāa University Hospital during a 6 months period. Participation in the study was voluntary after an informed consent was obtained.

Patients:
Patients were classified into 2 groups; Group I: Sixty five patients who were suffered from HCV related chronic liver diseases and Group II: Thirty patients who were diagnosed as HCV related hepatocellular carcinoma. Patients were evaluated for the presence of liver disease by means of clinical and biochemical assessments. Chronic hepatitis C was diagnosed by the elevated liver enzymes for more than 6 months and presence of HCV antibodies. The hepatocellular carcinoma (HCC) was diagnosed by the presence of high level of α-fetoprotein ultrasonography and histopathological picture of percutaneous liver biopsy.

All patients were subjected to full history taking, careful clinical examination, liver function tests, upper endoscopy and sigmoidoscopy for diagnosis of schistosomiasis and ultrasonography and were classified according to Child classification. Patients with end stage renal disease, blood disease or receiving immune suppressive medications (corticosteroids–cytotoxic drugs for any chronic disease) were excluded. None of the patients selected had HIV infection or receive interferon therapy or famvidin.

Methods:

Serological assays
All serum samples were tested for serum alanine transaminase (ALT; upper normal limit UNL 42 IU/L) and aspartate transaminase (AST; UNL 37 IU/L) using standard autoanalyser (Hitachi 911) and assays of Dia SYS international (Germany). Hepatitis B markers (HBsAg, Anti HBe (IgM & total) and HBeAg) were tested using commercially available enzyme linked immunoassays (ELISA; Abbott-Murex laboratories, North Chicago IL, USA). Antibodies to HCV (anti-HCV) were detected using a standard third generation ELISA test (Murex anti-HCV, version 4.0).

Molecular assays:
Nucleic acid extraction: DNA/RNA was extracted from 200 µl serum using the High Pure Viral Nucleic acid assay (Roche Diagnostics, GmbH-Boehringer, Germany) following the manufacturer’s instructions. DNA/RNA was eluted from filters with 50 µl of elution buffer.

HBV-DNA analysis:
All DNA extracts were analyzed for HBV genomes with two different nested polymerase chain reaction (PCR) assays to detect surface “s” and core “c” genes according to the methods described by Cacciola et al., (8). Briefly, 100 µl of reaction mixture containing 10µl of extracted DNA, 50 µl Taq PCR Master Mix (QIAGEN, Germany) and 20 pmol each of the oligonucleotide primers (QIAGEN-OPERON Germany) HBV1 and HBV2 for the "s" gene and primers HBV3 and HBV4 for the "c" gene. Amplification conditions were 95°C for 5 minutes for one cycle then 35 cycles each consisting of denaturing for 30 seconds at 94°C, annealing for 45 seconds at 56°C and extension for 1.5 minutes at 72°C and a final extension for 7 minutes after the last cycle in an automated thermal cycler (T1-Biometra). A second round PCR amplification was performed with 10 µl from the product of the first reaction and the inner primers were HBV5 and HBV6 for the "s" region and HBV7 and HBV8 for the "c" region(8).

Patients who were positive for only one of the two regions examined were restested by single-step or nested PCR procedures with the use of sets of oligo-
nucleotide primers (HBV9, HBV10, HBV11 and HBV12) encompassing the viral “x” gene. The primers used were complementary to the conserved regions of HBV genotype D at the following positions (from 5’ to 3’): HBV1, 61-81; HBV2, 1004-985; HBV3, 1778-1800; HBV4, 2483-2464; HBV5, 154-174; HBV6, 839-819; HBV7, 1928-1946; HBV8, 2391-2372; HBV9, 968-986; HBV10, 1951-1931; HBV11, 1266-1283; and HBV12, 1804-1784 (8). The limit of sensitivity of the nested PCR method was 1x10⁶ pg of cloned HBV-DNA. The amplification products were visualized on an ethidium bromide-stained 1.5% agarose gel in 1x Tris borate EDTA buffer. DNA molecular size markers of 100 bp ladder (Roche Diagnostics, GmbH, Germany) were also run in each gel, separation bands were visualized and photographed under UV illumination. Full precautions were taken and a negative control was included with each set of samples.

HCV RNA analysis

RT-PCR was performed as described by Cha et al. (11). Ten µl of extracted RNA was added to 26 µl of DEPC water, 10 µl buffer 5x (250 mM Tris-HCl pH 8.3, 375 mM KCl, 10mM MgCl₂, 50mM DTT), 2 µl random primers (0.01 µg/ml), 0.5 µl of DNTPs (10mM), 1 µl of RNase inhibitor (25u/ml) and 0.5 µl of reverse transcriptase enzyme (200 u/ml) Promega- USA The reaction took place in a thermocycler for 45 min at 37°C, and for 5 minutes at 95°C. Five µl of cDNA was then subjected to nested PCR according to Cha et al., (11). The reaction was performed in a 50 µl total reaction volume. The first round used 50 pmole/µl of antisense primer (5’-GGT GCA CGG TCT ACG AGA CCT C-3’) and sense primer (5’-AAC TAC TGT CTT CAC GCA GAA-3’). Amplification conditions were 95°C for 5 min., 95°C for 1 min., 54°C for 1 min., and 72 °C for 2 min. for 34 cycles to be extended to 72°C for 7 min. The second round PCR used 50mole /µl of anti-sense primer (5’-GGT GCA CGG TCT ACG AGA CCT C-3’) and sense primer (5’-AAC TAC TGT CTT CAC GCA GAA-3’) with different amplification conditions as follows: 95°C for 5 min, 94°C for 1 min, 50 °C for 1 min., and 72 °C for 2 min. for 34 cycles to be extended to 72°C for 7 min. The amplified product (169 bp) was analyzed in 2% agarose gel electrophoresis as mentioned before.

Statistical analysis:

Data entry and statistical analysis was performed using SPSS under windows, version 9. Chi square test was used in order to detect the difference in proportions between groups. Fisher's exact test was used when there was a cell in the 2x2 table with an expected frequency below 5 and t-test was used to compare between two means. When there was a highly skewed distribution of continuous variables, nonparametric, tests as Mann whitney test was used. P Value < 0.05 was considered significant.

RESULTS

Ninety five patients with HCV related chronic liver disease were included in this study. Thirty of them were suffering from HCC. All the studied patients were anti-HCV positive, where 47/95 (49.5%) of them were HCV RNA positive. Forty five (47.3%) of the patients were of Child classification type A, 19(20%) were of Child classification type B and 31 (32.6) were of type C. ALT and AST were increased by the double of the normal value in all patients. α FP was elevated in HCC patients.

HBV markers were evaluated in all patients for the presence of dual infection, HBsAg was detected in 25/95 (26.3%) (Overt HBV infection), Total anti-HBc was detected in 52/83 (62.65%), only one of them was also positive for anti-HBc IgM while HBVAg was detected in 2 (3.1%) patients. HBV DNA was detected in 41/95(43.1%) patients.

Nested PCR was performed to detect HBV DNA in three regions; c, s and x. Region was detected in sera of 39/95 (41%) patients , s region was detected in 12/95 (12.56%) and only one serum sample was positive for x region, Figure (1).

When HCV-CLD (group I) patients were compared with those having HCC (group II), it was found that Child classification type A is significantly increased in the first group while the second group was significantly categorized in type C (P<0.005). The mean of ALT & AST levels were also significantly elevated within this group (P<0.001&P<0.01) respectively. HCV RNA as well as HBV markers were comparable in
both groups. On the other hand, HBV DNA is significantly detected in group I (P< 0.0005) for c region. Moreover, HBV DNA positive / HBsAg negative (occult HBV infection) was significantly prevalent in group I (p<0.001), Table (1). Occult HBV infection was detected in 30/95 (31.57%) and was found to be significantly increased in those who were HCV RNA positive rather than in HCV RNA negative patients (P< 0.005), Figure (2) & Table (2). Both also demonstrated that total anti-HBe is present in absence of HBsAg in 22/65 (33.8%) in group I and in 11/30 (36.6%) in group II, even more, it highlighted the possibility of detection of total anti-HBe as a sole marker indicating HBV infection in such patients. It was the only marker denoting HBV infection in 15/95 (15.78%) patients, 7 of them were in group I and 8 were in group II. Statistical analysis of the clinical and laboratory findings of overt vs occult HBV infected patients, revealed that occult infection is more prevalent in HCV related CLD (group I) rather than HCC patients (group II), P<0.005, Table (2). ALT and AST were comparable in both groups. HCV viraemia was significantly detected in occult rather than overt infection P<0.05. The percentage of patients showing HBV viraemia for "c" region was also significantly detected among patients with occult infection (100%) compared to those with overt infection (32%) P<0.0005 Table (3).

Table 1: Statistical analysis of the Clinical and Laboratory findings of the studied patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hepatitis C CLD</th>
<th>HCC</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age± SD</td>
<td>50.8±9.9</td>
<td>56.2±8.9</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>28 (43.1)</td>
<td>23 (76.7)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>37 (56.9)</td>
<td>7 (23.3)</td>
<td></td>
</tr>
<tr>
<td>Child classification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>41 (63.07)</td>
<td>4 (13.3)</td>
<td>&lt;0.0005*</td>
</tr>
<tr>
<td>B</td>
<td>14 (21.5)</td>
<td>5 (16.7)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>10 (15.3)</td>
<td>21 (70.0)</td>
<td></td>
</tr>
<tr>
<td>Mean ALT</td>
<td>60.6±37.2</td>
<td>92±50.2</td>
<td>0.001*</td>
</tr>
<tr>
<td>Mean AST</td>
<td>72.0±48.2</td>
<td>87.43±36</td>
<td>0.01*</td>
</tr>
<tr>
<td>HCV Abs + ve</td>
<td>65 (100)</td>
<td>30 (100)</td>
<td>0.23</td>
</tr>
<tr>
<td>HCV RT- PCR + ve</td>
<td>34 (52.3)</td>
<td>13 (43.3)</td>
<td>0.66</td>
</tr>
<tr>
<td>HBsAg + ve</td>
<td>14 (21.5)</td>
<td>11 (36.7)</td>
<td>0.35</td>
</tr>
<tr>
<td>HBeAg (1gM)**</td>
<td>1 (1.5)</td>
<td>0 (0.0)</td>
<td>0.71</td>
</tr>
<tr>
<td>HBeAg (total)***</td>
<td>33/56 (58.9)</td>
<td>19/27 (59.3)</td>
<td>0.4</td>
</tr>
<tr>
<td>HBV -PCR (s region) + ve</td>
<td>2 (3.1)</td>
<td>0 (0.0)</td>
<td>0.53</td>
</tr>
<tr>
<td>HBV-PCR (c region) + ve</td>
<td>11 (15.9)</td>
<td>1 (3.3)</td>
<td>0.069</td>
</tr>
<tr>
<td>HBVDNA+ve/HBsAg negative****</td>
<td>36 (55.38)</td>
<td>3 (10%)</td>
<td>&lt;0.0005*</td>
</tr>
<tr>
<td></td>
<td>27 (41.5%)</td>
<td>3 (10%)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

*Significant
**This patient was also HBeAg (total) and both markers are the only indicators of HBV infection
***HBeAg (total) was tested in 83 patients only
****Occult HBV infection
Total HBV-DNA viraemia: 41

C region: 39
S region: 12

X region: 1

Figure 1: Prevalence of HBV- DNA (different regions) in sera of the studied patients

95 HCV CLD Patients

47 +ve HCV PCR (49.5%)
48 -ve HCV PCR (50.5%)

11 +ve HBsAg (23.4)
36 -ve HBsAg (76.6%)
14 +ve HBsAg (29.2%)
34 -ve HBsAg (70.8%)

P = 0.007

Table 2: Statistical analysis of HCV RT- PCR, HBV- PCR and HBV markers among the studied groups

<table>
<thead>
<tr>
<th></th>
<th>HCV-CLD(65)</th>
<th>6**</th>
<th>3**</th>
<th>18*</th>
<th>7</th>
<th>0</th>
<th>5**</th>
<th>9*</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBeAb –total(33)</td>
<td>5</td>
<td>2</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>3</td>
<td>7***</td>
<td></td>
</tr>
<tr>
<td>HCC (30)</td>
<td>1**</td>
<td>1**</td>
<td>3*</td>
<td>8</td>
<td>1**</td>
<td>8**</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>HBeAb-total(19)</td>
<td>-</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>1</td>
<td>6</td>
<td>-</td>
<td>8***</td>
<td></td>
</tr>
</tbody>
</table>

* Patients with occult HBV infection
** Patients with overt HBV infection
*** HBeAb –total is the only HBV marker in 7 HCV-CLD & in 8HCC patients
† P value was calculated using Fisher's exact test comparing results of PCR for HCV &HBV when HBsAg was positive
‡ P value was calculated using Chi square test comparing results of PCR for HCV &HBV when HBsAg was negative
Table 3: Statistical analysis of the Clinical and Laboratory findings in patients with occult and overt HBV infection.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total n=55</th>
<th>Occult HBV infection N=30 (%)</th>
<th>Overt HBV infection N=25 (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ± SD</td>
<td>55</td>
<td>50.6±10.3</td>
<td>51.6±9.8</td>
<td>0.691</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>33</td>
<td>17(56.7)</td>
<td>16(64.0)</td>
<td>0.783</td>
</tr>
<tr>
<td>Females</td>
<td>22</td>
<td>13(43.3)</td>
<td>9(36.0)</td>
<td></td>
</tr>
<tr>
<td>CHILD classification</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>27</td>
<td>17(56.7)</td>
<td>10(40.0)</td>
<td>1.159</td>
</tr>
<tr>
<td>B</td>
<td>11</td>
<td>7(23.3)</td>
<td>4(16.0)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>17</td>
<td>6(20.0)</td>
<td>11(44.0)</td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HCC</td>
<td>14</td>
<td>3(10.0)</td>
<td>11(56.0)</td>
<td>0.004*</td>
</tr>
<tr>
<td>HCV related CLD</td>
<td>41</td>
<td>27(90.0)</td>
<td>14(44.0)</td>
<td></td>
</tr>
<tr>
<td>Mean ALT</td>
<td>55</td>
<td>65.2±45.4</td>
<td>75.5±51.9</td>
<td>0.641</td>
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<tr>
<td>Mean AST</td>
<td>55</td>
<td>79.7±55.6</td>
<td>73.1±46.9</td>
<td>0.671</td>
</tr>
<tr>
<td>Mean α FP</td>
<td>55</td>
<td>1176.7±642.2</td>
<td>3645.7±6609.2</td>
<td>0.741</td>
</tr>
<tr>
<td>HCV RT-PCR + ve</td>
<td>32</td>
<td>21(70.0)</td>
<td>11(44.0)</td>
<td>0.052*</td>
</tr>
<tr>
<td>HBsAg + ve</td>
<td>25</td>
<td>0.0(0.0)</td>
<td>25(100.0)</td>
<td>&lt;0.0005*</td>
</tr>
<tr>
<td>HBeAb + ve</td>
<td>37</td>
<td>18(66.7)</td>
<td>19(82.6)</td>
<td>0.200</td>
</tr>
<tr>
<td>HBeAg + ve</td>
<td>2</td>
<td>0(0.0)</td>
<td>2(8)</td>
<td>0.274</td>
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<tr>
<td>HBVPCRc + ve</td>
<td>38</td>
<td>30(100)</td>
<td>8(32)</td>
<td>&lt;0.0005*</td>
</tr>
<tr>
<td>HBVPCRs + ve</td>
<td>12</td>
<td>9(30.0)</td>
<td>2(12.0)</td>
<td>0.112</td>
</tr>
</tbody>
</table>

*Significant
N.B. 40 patients were negative for both HBsAg & HBV-DNA

DISCUSSION

Although occult HBV infection has been identified in patients with chronic hepatitis C liver disease, their prevalence and clinical significance are not known yet (8). Brechot et al. (12) provided very strong evidence that occult HBV infection exists and that most cases are related to very low levels of HBV rather than to HBV mutants that do not express or produce aberrant surface proteins and therefore are undetected by standard testing.

As occult HBV infection is usually associated with very low level of vireamia, the question is whether the presence of such HBV low vireamia will lead to progressive liver disease or not. Conjeevaram and Lok, (13) mentioned that available data suggest that the likelihood is extremely low although definitive data are lacking.

HBV and HCV infections are common in Egypt, a National Survey of HBV and HCV was carried out in 1996/1997 to study the prevalence of HBV/HCV in the general population of ten governorates in Egypt, revealed that it was 4.5% and 12% for HBV and HCV respectively. It is well known that blood borne HBV infection is more frequent than HCV due to HBV higher concentration in patients’ blood. This decline of HBV CLD among Egyptians could not be explained by factors such as vaccination because Egypt started its compulsory vaccination program for children in 1992 and also because vaccination does not cure already infected adults, so it is difficult to conceive how a single environment like the Egyptian environment promotes infection with one virus and suppress infection with other previously quite prevalent if the two viruses have the same mode of transmission. So the
The aim of this work is to determine the prevalence of occult HBV infection in patients with HCV related liver disease and to spot lights on its importance on the clinical outcomes of the infected patients.

Because HBV-DNA detection is the key for diagnosis of occult HBV infection, the type of assay used and its sensitivity must be specified(13). The sensitivity of PCR assays for HBV-DNA in studies of occult HBV infection varies from 10 to 10³ copies/mL(14).

Ninety five patients with HCV related chronic liver disease were included in this study. Thirty of them were suffering from HCC. Nested PCR methods were used to detect HBV-DNA in three different regions(s, c &x) of the HBV genome. The limit of sensitivity of the nested PCR method was 1x10⁶ pg of cloned HBV-DNA. The overall occult HBV infection was detected in 30/95(31.57%) patients. El Gohary et al. (15) studied the prevalence of occult HBV infection in acute hepatitis of unidentified aetiology and in HCV CLD patients. They found occult HBV infection in 66.6% and 38.9% in both groups respectively and concluded that occult HBV infection is highly prevalent in Egypt and may have clinical significance. Scully et al., (16) stated that geographic differences in the prevalence of occult HBV infection are most likely related to the endemicity of HBV infection. He further added that, it also depends on the population studied, being more common in patients with CLD. In this study, a high significant prevalence of occult HBV infection was among HCV related CLD patients rather than those with HCC. However, Matuszaki et al. (17) confirmed that beside HBV, other environmental and host factors may also be associated with the pathogenesis of HCC.

Using PCR amplification, most studies (Fukuda et al., (7), Cacciola et al., (8) & Berger a et al.,(18)) demonstrated the presence of HBV-DNA genome in 22% to 87% of the patients with HBsAg negative and HCV RNA positive test. The frequency of detectable HBV-DNA was significantly higher in patients with chronic HCV related liver disease than in those with non HCV related liver disease(8). Therefore, concomitant HCV and occult HBV co-infection is a frequently encountered clinical entity in patient with chronic HCV infection. The current study which showed a significant increased prevalence of occult HBV in those who were HCV RNA positive rather than in HCV RNA negative patients was in accordance with these results.

The clinical impact of HCV and Occult HBV co-infection remains controversial. Cacciola, et al (8) have shown that patients with HCV and occult HBV co-infection had a significantly higher incidence of cirrhosis than those with HCV infection alone and occult HBV co-infection was also associated with higher level of ALT and histological activity(19,20). However, other studies suggested that the incidence of liver related complications in these patients appears comparable to that in patients with HCV infection alone(21,22). The current study goes with the later opinion as no significant difference was detected between patients with occult or over HBV infection as regard to liver enzymes or Child classification (P>0.05). Even more Child classification is more worsen and down graded together with significant higher levels of liver enzymes in the HCC patients group which showed low occult HBV prevalence. Kao et al.,(23) explained the long duration and the immune tolerance to HBV to be the cause which modulates the influence of occult HBV infection on the histological damage in chronic hepatitis C patients.

In the current study, assessment of HBV-DNA in three different region of the genome demonstrated high significant detection of the "c" region. It was also highly significant in HCV-CLD patients and in those who were suffering from occult HBV infection. Uchida et al., (20) has successfully transfected human hepatoma cell line by full length HBV-DNA genome cloned from patients with HCV and occult HBV infection indicating the replicating capacity of HBV in these patients. It appears that only a small portion of these patients might have the entire HBV genome in liver and serum as determined by different HBV oligo-primers(24). It was claimed that, the higher frequency of fragmented HBV genome in liver tissue indicates possible HBV-DNA integration into the cellular DNA in these patients(5). Likewise, the presence or circulating fragmented HBV-DNA may be secondary to HBV subgenomic expression of the integrated HBV-DNA.
In this study, total HBV Anti-core was detected in HCV related CLD as well as HCC patients (58.9% & 59.3%) respectively. It was positive in 33 HBsAg negative patients, of them 18/30 (60%) were occult infection. Scully et al.,(16) reported that patients with serum hepatitis B core antibody positivity are much more likely to have occult HBV-DNA present than patients with only HBsAb positivity or no markers at all. Also Lai et al.,(25) argued the disappearance of HBsAg and in some cases of all HBV markers despite the persistence of HBV infection to mutation or integration of HBV-DNA sequence that may alter expression of HBV proteins, resulting in undetectable HBsAg and possible escape of host immune response. Persistent HBV infection may be maintained by extra hepatic HBV replication, such as HBV infection of peripheral blood mononuclear cells or other lymphoid tissues.^(26^)

The current study emphasized that occult HBV infection is significantly increased in HCV-RNA positive than in those who were HCV-RNA negative. This could be explained by more than one mechanism; Coinfection of HBV with HCV may lead to low rate of replication, due to mutual interference between both viruses as Brechot et al.,(12) Jeanet al.,(27) Sagnelli et al.(4) showed an inhibitory effect of HCV on HBV replication. This inhibitory activity was also reported by Sheen et al.(28) who showed a rate of HBsAg clearance 2.5 times more in HBsAg/anti HCV positive cases than in those with HBV infection alone. However follow up of HBV-DNA by PCR has shown a variable prevalence of occult HBV infection in this category of patients,(21) indicating a fluctuating HBV viraemia during occult HBV infection.^(8^)

The possibility of persisted HBV infection in anti-HBc positive individuals 18/30 (60%) has been supported by previous studies showing that traces of HBV are often detectable in the blood for many years after clinical recovery from acute hepatitis, despite the presence of antibodies against HBV and HBV-specific cytotoxic T-lymphocytes in serum.^(29,19^)

These findings indicate an active HBV replication and release of HBV virus into the circulation of the patients with concomitant HCV and occult HBV confection, a frequently encountered clinical entity in patients with chronic HCV infection. These results point to that hepatitis B virus infection is not vanishing but it is rather in an occult or mutant form. However, hepatitis B occult infection frequency depends on the prevalence of HBV infection in the population and sensitivity of both tests; HBsAg and HBV DNA assays. However, most PCR assays including commercially available assays are not standardized.^(14^)

The critical question is whether or not occult B infection is infectious. Conjeevaram and Lok,(13) emphasized that transmission of HBV infection has been documented from HBsAg-negative, anti-HBc-positive blood and organ donors. Also Allain,(6) showed that by transfusion all forms have been infectious in immune-compromised individuals while in immune-competent recipients there is no evidence that anti HBs containing components (at low titer) are infectious.

World wide, HCC is one of the most common malignancies associated with poor prognosis.^(30^). In Egypt there was a remarkable increase of the proportion of HCC among CLD patients from 3.0% to 7.2% over a decade.^(31^) Epidemiologic studies and laboratory investigations have established that HBV is a major etiologic factor of HCC.^(32^) Early studies have revealed that a higher prevalence of anti-HCV antibody was more associated with HCC.^(33,34^)

In this study, overt HBV infection was detected in 11/30 (36.7%) of the HCV-related HCC patients. Two of them were HCV-RNA positive. On the other hand, total Anti-c was detected in 19/27 (59.3%), three of them were showing occult infection. Total HBV anti-core was the only marker denoting HBV infection in 8 HCC patients who were also HCV-RNA negative. These results are in agreement with those reported by Kubo et al.^(35^) and Marusawa et al.^(36^) who found that the prevalence of anti-HBC and or anti-HBs varies from 50 to 90% among patients with anti-HCV positive HCC. Also, Geo et al.^(37^) suggested a tight association of HBV with HCC and that HBV has a dominant role in causing HCC, as they found about 11.8% of HCC patients being HCV-related were coinfected with HBV. These findings were in agreement with other studies of Ohaba et al.^(38^), Tanaka et al.^(39^) & Monasaki et al.^(40^). These results indicated that anti-HBc...
rather than occult HBV infection is associated with a worse outcome in HCV infected patients\(^{(14,41)}\). Moreover, anti-HBe screening identifies most of occult HBV but not all, over time, antibody markers may become undetectable leaving HBVDNA as the only marker of infection\(^{(41)}\).

CONCLUSION

This study demonstrated that occult HBV infection represents a special form of HBV infection that is frequently detected among HCV infected patients increasing the prevalence of HBV infection than it is really known. Routine serological profiles are not always reliable in determining status of HBV infection. Standardized definition and diagnostic criterion of occult HBV infection are needed for future research to determine the prevalence and clinical significance of occult HBV infection and the role of antiviral therapy.

Prospective studies are also required to establish the relative risk of HCC among individuals with HBsAg-negative chronic liver disease, with or without HBV-DNA detection and HCV co-infection. HBV DNA test should be only performed in HBsAg negative and anti-HBe positive patients with continuous chronic hepatic inflammation, and also in anti-HCV positive patients.

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The study aimed to investigate the role of pyogenic bacteria in acute liver abscesses among Egyptian patients. A total of 59 patients were included in the study, and the results were analyzed for the presence of bacteria in the abscess fluid.

The study found that the most common bacteria isolated were Escherichia coli (27.1%) and Staphylococcus aureus (17.9%). The findings also showed a significant correlation between the presence of pyogenic bacteria and the severity of the liver abscesses (P < 0.05).

These results highlight the importance of considering pyogenic bacteria in the management of acute liver abscesses and support the use of antibiotics as a therapeutic option.