Persistent Elevation of Liver Enzymes during Pegylated Interferon Therapy of Chronic Hepatitis C Virus: Role of Occult Hepatitis B

Mohamed H Emara, Ehab M Darweish, Ahmed S Bihery, Salem Y Mohamed, Heba F Pasha

ABSTRACT

AIM: Lack of liver enzymes normalization may discourage patients with chronic HCV from continuing therapy and may worry the clinician about treatment outcomes, raising the suspicion about the presence of concomitant causes of liver damage. The decision of discontinuing treatment solely on the basis of elevated liver enzymes in patients who are HCV-RNA-negative would result in a substantial proportion of patients not being cured of infection. We conducted this study to test the assumption that persistent liver enzymes elevation in patients with chronic HCV under pegylated interferon therapy who have achieved HCV clearance may be due to an underlying occult hepatitis B virus infection.

METHODS: Seventy six chronic HCV patients under treatment with pegylated-interferon/ribavirin therapy were included. Group I (n=38); patients with persistent liver enzymes elevation despite therapy, while group II (n=38); patients with persistently non elevated liver enzymes. All patients were not viremic for HCV at the time of examination to hepatitis B virus. All patients were investigated for liver enzymes. All patients were not viremic for HCV at the time of therapy, while group II (n=38); patients with persistent liver enzymes elevation despite pegylated interferon/ribavirin (RBV) that proved superior to standard interferon alpha (IFN) and ribavirin (RBV) that proved superior to standard interferon alpha and ribavirin.(8,9). Elevated aminotransferases are considered a sign of hepatocellular damage due to their high hepatic concentration, and alanine aminotransferase (ALT) is more specific for liver damage than aspartate aminotransferase (AST)(10). Lack of ALT and AST normalization may discourage patients from continuing therapy and may worry the clinician about treatment outcomes, raising the suspicion about the presence of concomitant causes of liver damage. The decision of discontinuing treatment solely on the basis of elevated aminotransferases in patients who are HCV-RNA-negative would result in a substantial proportion of patients not being cured of infection.(7).

CONCLUSIONS: Occult hepatitis B virus infection is not a cause of persistent elevation of liver enzymes during pegylated-interferon therapy of chronic HCV.

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Key words: Occult hepatitis B; Chronic HCV; Liver enzymes; Pegylated-interferon

presence of HBV DNA, in serum and/or the liver tissue without detectable HBsAg with or without anti-HBc or anti-HBs outside the pre-seroconversion window period\(^\text{[11]}\). OBI may contribute to liver inflammation through episodes of increased viral replication, increased immune activity and subsequent liver injury\(^\text{[12]}\).

In this paper, the authors assume that patients with chronic HCV infection who have achieved HCV clearance and are concomitantly infected with OBI can experience persistent liver enzymes elevation during HCV therapy due to flare of HBV infection.

**METHODS**

This study was conducted in Al-Ahrar General Hospital (local treatment centre for Hepatitis C virus), Sharkia Governorate, Egypt in the period from April 2011 to March 2012 and included 76 chronic HCV patients under Peg-IFN/RBV therapy. The diagnosis of HCV was done by detection of anti-HCV antibody and confirmed by HCV RNA and they were all candidates to begin combination therapy according to the modified guidelines of the national Committee for Control and Prevention of viral Hepatitis "C" in Egypt, with the following criteria:

**Inclusion criteria**

1- Age \(\geq\) 18 years.
2- White blood cell (WBC) count > 3500/mm\(^3\).
3- Neutrophil count>1 500/mm\(^3\).
4- Platelets >8 5000/mm\(^3\).
5- Prothrombin time <2 seconds above upper limit of normal (ULN).
6- Direct bilirubin 0.3 mg/dL or within 20% ULN.
7- Indirect bilirubin 0.8 mg/dL or within 20% ULN.
8- Fasting blood sugar 115 mg or within 20% ULN.
9- Albumin > 3.5 gm/dL.
10- Serum creatinine within normal limit (WNL).
11- TSH WNL.
12- HBsAg is negative.
13- Anti-nuclear antibody (ANA) <1:160.
14- Positive anti-HCV and HCV RNA.
15- If diabetic, HB A1C <8.5%.
16- Alpha Feto-Protein (AFP) <100 IU/mL.
17- If AFP is abnormal (>100 IU/mL) triphasic C.T. is normal.
18- Female patient should practice adequate contraception.
19- If ALT is normal (<1 U/L) while anti-HBs is negative.
20- Signed written informed consent for the study.

**Exclusion criteria**

Exclusion of:
1- Any other cause of liver disease other than HCV (by laboratory and liver biopsy).
2- Co-infection with HBV.
3- Haemochromatosis.
4- Alpha-antitrypsin deficiency.
5- Wilson's disease.
6- Autoimmune disease (by ANA).
7- Alcoholic liver disease.
8- Decompensated liver disease.
9- Hypersensitivity to Peg-IFN or ribavirin.
10- Pregnancy or breast feeding.
11- Poorly controlled diabetes.
12- Clinically significant retinal abnormalities.
13- Obesity –induced liver disease.
14- Drug-induced liver disease.

All the studied patients were subjected to the following:
I. Detailed history taking.
II. Thorough clinical examination.
III. Routine investigations; liver function tests, kidney functions, complete blood counts. Liver enzymes measured as times of ULN from reference values.
IV. Revision of the pre-enrollment laboratory investigations with special concern to liver functions, kidney functions, complete blood count, serum HCV RNA.
V. Liver biopsy for histological staging and grading of chronic HCV: Hepatitis grading and staging were evaluated according to the META VIR scoring system.
VI. Body mass index (BMI) assessment.
VII. Serodiagnosis of HBV: anti-HBc total antibodies were detected by a rapid immunoassay (Gold Colloidal Conjugate Membrane, Cal-Tech Diagnostics, INC.- California, USA) while anti-HBs antibodies were determined by the electro-chemi-luminescence immunoassay technique using commercially available kits (Cobas e 411 analyzer, Hitachi, Japan).
VIII. HBV DNA examination.

The assay and results were performed and interpreted by investigators who were blinded to the patients' baseline characteristics, HBV serology, stage of liver biopsy and clinical outcome. HBV DNA examination was done by COBAS® AmpliPrep/COBAS® TaqMan® HBV Test (Roche Diagnostics, Switzerland), it is a real time quantitative PCR technology. The concentration of HBV DNA in EDTA-plasma that can be detected with a positivity rate of greater than 95%, is 12 IU/mL or lower while its specificity is 100%.

**Treatment regimens and follow up**

The dose of Peg-IFN alpha-2a is 180 ug subcutaneously around the umbilicus once per week together with RBV, using 1000 mg/d for those \(\leq 75\) kg in weight and 1200 mg/d for those >75 kg in weight; the dose of Peg-IFN alph-2b is 1.5 ug/kg body weight subcutaneously around the umbilicus once per week together with RBV at dose of...
Data were checked, entered and analyzed using SPSS (Statistical Package for the Social Sciences) version 15. Data were expressed as mean±SD for quantitative variable, number and percentage for qualitative one. Chi-squared ($X^2$) or Fisher exact, $t$ test and paired $t$ test were used when appropriate. $P<0.05$ was considered significant.

**RESULTS**

The base line characteristics of both groups are shown in table 1. All patients of this study were HBsAg negative. Patients in group I had both ALT and AST elevated throughout the study period with mean elevation of 3.1 and 2.76 times the ULN from the reference values respectively. None of the cases in persistent liver enzymes elevation group had levels >7 times the reference values.

Regarding the markers of HBV infection (Table 2); none showed statistical significance in relation to the persistent liver enzymes elevation of 3.1 and 2.76 times the ULN from the reference values respectively. None of the cases in persistent liver enzymes elevation of 3.1 and 2.76 times the ULN from the reference values respectively. None of the cases in persistent liver enzymes elevation group had levels >7 times the reference values.

![Figure 1 Hepatic Fibrosis in both groups.](image1)

![Figure 2 Hepatic necroinflammatory activity in both groups.](image2)

800 mg/d for those weighting <65 kg; 1000 mg for those >65 kg to 85 kg; 1200 mg for those >85 kg to 105 kg; and 1400 mg those >105 kg.

**Patient Monitoring**

Patients were assessed at weeks 0, 1, 2 and 4 of treatment and thereafter monthly. At each visit, laboratory tests were performed including serum ALT and AST, bilirubin, complete blood count, and serum creatinine. Body weight and symptom checklist were recorded at each visit and dose modifications to the Peg-IFN or RBV were made when appropriate. Qualitative HCV-RNA was determined at week 12, week 24. Quantitative serum HCV-RNA was determined at baseline and at week 12.

**STATISTICAL ANALYSIS**

Data were checked, entered and analyzed using SPSS (Statistical

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Table 1 Characteristics of the studied patients.

<table>
<thead>
<tr>
<th></th>
<th>Group I (N=38)</th>
<th>Group II (N=38)</th>
<th>$t$</th>
<th>$P$</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
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<tr>
<td>X ± SD</td>
<td>43±10.9</td>
<td>41±8.2</td>
<td>0.38</td>
<td>&gt;0.5</td>
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<tr>
<td>Range</td>
<td>20-59</td>
<td>28-59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>28</td>
<td>28</td>
<td>1.0</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X ± SD</td>
<td>27.3±3.7</td>
<td>27.2±2.9</td>
<td>0.89</td>
<td>&gt;0.5</td>
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<tr>
<td>Range</td>
<td>22-35</td>
<td>22-33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>23</td>
<td>22</td>
<td>0.26</td>
<td>&gt;0.5</td>
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<tr>
<td>Active</td>
<td>7</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-smoker</td>
<td>8</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT (ULN)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>X ± SD</td>
<td>3.1±1.69</td>
<td>1±0.0</td>
<td>7.76</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Range</td>
<td>1.5-7</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AST (ULN)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X ± SD</td>
<td>2.76±1.1</td>
<td>1±0.0</td>
<td>8.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Range</td>
<td>1.5-6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV RVA</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(IU/ml)&lt;10$^3$</td>
<td>6.7±7.4</td>
<td>3.6±5.2</td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.017-22.3</td>
<td>0.009-22.2</td>
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<td></td>
</tr>
<tr>
<td>Median</td>
<td>2.08</td>
<td>1.64</td>
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</tbody>
</table>

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Table 2 HBV markers in both groups.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Anti-HBs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>32</td>
<td>84.2</td>
<td>0.76</td>
</tr>
<tr>
<td>Positive</td>
<td>6</td>
<td>15.8</td>
<td></td>
</tr>
<tr>
<td>Anti-HBc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>32</td>
<td>84.2</td>
<td>0.76</td>
</tr>
<tr>
<td>Positive</td>
<td>6</td>
<td>15.8</td>
<td></td>
</tr>
<tr>
<td>HBV DNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>36</td>
<td>91.4</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
<td>8.6</td>
<td></td>
</tr>
</tbody>
</table>

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Table 3 HBV DNA in relation to anti-HBc and anti-HBs.

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV DNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HBc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>49(64.5%)</td>
<td>10(13.1%)</td>
<td>62</td>
</tr>
<tr>
<td>Positive</td>
<td>10(13.1%)</td>
<td>4(5.3%)</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>59(77.6%)</td>
<td>14(18.4%)</td>
<td>76</td>
</tr>
</tbody>
</table>
while on Peg-IFN/RBV therapy. Group I had advanced hepatic fibrosis (F3, F4) in comparison with group II \(p=0.001\), the same do occur for histologic activity, where patients in group I had advanced activity (A2, A3) than group II \(p=0.001\). The same is noticed with base line HCV RNA, where patients with group I had higher base line HCV viral load than group II \(p=0.039\).

**DISCUSSION**

Occult HBV infection has frequently been identified in patients with HCV-related chronic hepatitis. Considerable data suggested that occult infection may contribute to chronic liver damage, poor response to antiviral therapy, and the development of HCC.

Liver enzymes flare due to OBI has been previously reported in persons with HCV infection in international and Egyptian studies. The ALT levels are usually mildly higher in OBI and HCV co-infection and seems to be either similar or mildly raised, than in HCV mono-infection patients. Fujiwara et al found no correlation between HBV DNA and enzymes elevation, this agrees with our results. In the other hand several investigators demonstrated significant relation between flares in liver transaminases and OBI. These notions support the hypothesis that OBI replication is a cause of hepatocyte injury.

OBI occurs in 3.9% of the studied sample in this study and this is in agreement with previous rates reported from local Egyptian studies as well as international studies. In the subgroup analysis OBI occurs in 5.2%, in the group of persistent liver enzymes elevation and this is much lower than rates reported in Egypt by Selim et al, who reported OBI in 63.3% of chronic HCV patients with ALT flares, this can be explained by the fact that OBI may be eradicated, if present, by the interferon therapy in our patients when compared to non treated patients in their study.

OBI is not a cause of persistent liver enzymes elevation in Egyptian chronic HCV patients under interferon therapy, where in this study 2 patients with detectable serum HBV DNA were detected in group I and one patient in group II. Also serological markers of HBV infection (anti-HBs, anti-HBc) were not associated with persistent liver enzymes elevation.

The observation that HBV DNA positivity cannot be predicted by serological markers is a finding of this study and agrees the previous findings in international as well as Egyptian studies.

The finding of occult HBV infection among anti-HBc positive persons supports the notion that occult HBV infection is frequently a late phase of overt chronic HBV infection or serologically recovered acute HBV infection. Another possible hypothesis for this finding is that HCV infection may block the circulating viral expression of HBV but anti-HBc in the serum and HBV DNA in the hepatocytes may persist. The reason for existence of occult HBV infection in anti-HBc negative patients is less clear. It is possible that some of these patients had transient acute HBV infection with clearance of HBsAg initially and decline of anti-HBc to undetectable levels over time. It is also possible that some patients are infected with HBV variants that do not express any HBV protein.

Hung et al observed that 13% of chronic HCV patients who obtained sustained virological response to standard interferon and RBV treatment showed persistently elevated ALT during treatment, whereas in the study by Zeuzem et al 41% of patients did not achieve ALT normalization until the end of therapy. These findings suggest that lack of ALT normalization is not necessarily associated with a decreased efficacy of treatment. This observation together with our findings should encourage chronic HCV patients under interferon therapy to continue therapy in spite of persistent liver enzymes elevation.

We knew that a point of weakness in this work is that HBV markers were measured only once, and a reciprocal viral dominance may alter the results, but this is compensated by the notice that all cases achieved some sort of HCV clearance (all were HCV RNA negative) at the time of examination to HBV DNA.

Persistent liver enzymes elevation in chronic HCV patients despite pegylated interferon therapy in this study seem to be related to hepatic pathology. In particular, advanced fibrosis (F3, F4) and activity (A3) seemed to play a role, where patients in group I had advanced hepatic fibrosis and histologic activity than group II. The same is applied to base line viral load, where persistent elevation of liver enzymes is more likely associated with higher baseline HCV viemia.

In conclusion OBI is not a cause of persistent liver enzymes elevation during Peg-IFN/RBV therapy of chronic HCV.

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