Study of the effects of antivirus therapy in patients with chronic liver disease due to hepatitis C virus infection

Abbreviated title: Efficacy of antiviral therapy in CLD

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Abstract

**Background:** Hepatitis C virus (HCV) infection is one of the leading causes of chronic liver disease (CLD). About 80% of those exposed to the virus develop a chronic infection. Hyperhomocysteinemia may develop in these patients although altered alanine amino transferase (ALT) enzyme levels are generally associated with damage to liver cells. The gold standard therapy for chronic hepatitis C patients is pegylated interferon combined with an anti-viral drug (ribavirin). The aim of the present study was to investigate the effect of this combined therapy and the diagnostic significance of homocysteine (Hcy) levels in addition to other parameters in these patients.

**Methods:** 532 CLD patients and 70 healthy controls were recruited for the study. All patients were subjected to laboratory investigations including HCV-RNA levels, complete blood cell counts, serum levels of homocysteine, ALT, ALP, lipid profile and liver ultrasonographic examination. The outcome of treatment with pegylated interferon α plus ribavirin treatment and sustained virologic response (SVR) was determined 6-9 months post-therapy.

**Results:** Hyperhomocysteinemia was found in 91.35 % of CLD patients. The difference in plasma Hcy concentrations reached statistical significance between the patient and control groups. ALT, cholesterol and triglyceride levels were found higher than normal in the patients group. After receiving a combined therapy for 24 weeks, 43.66 % patients showed an SVR; 30.98 % patients were non-responders while 25.35 % patients initially responded to therapy but again retrieved positive status of HCV infection six months post-therapy (relapse-cirrhotic). The mean levels of plasma Hcy, ALT and ALP were significantly reduced in the responders within 10 weeks of therapy when compared with non-responders and relapse-cirrhotic.

**Conclusion:** This study strengthens the evidence that supports the importance of standard interferon α plus ribavirin treatment in patients with chronic liver disease due to HCV infection and diagnostic significance of homocysteine levels in addition to other laboratory parameters.
Introduction

Chronic infection with hepatitis C virus (HCV) is one of the leading causes of chronic liver disease; about 170 million people worldwide are estimated to be infected. Hepatitis C infection causes acute symptoms in only 15% of patients exposed to HCV infection while about 80% patients develop chronic infection [1]. Chronic hepatitis C results in formation of high levels of free radicals in the liver cells, which put serious oxidative stress depleting protective antioxidants and eventually kill the liver cells. Chronic hepatitis C infection progresses very slowly and is marked by episodes of acute hepatitis characterized by liver inflammation and elevated ALT levels. A hepatitis screen is recommended for patients whereby the disease can be diagnosed by the presence of antibodies for hepatitis C or by the direct presence of the virus or viral products in the blood [2]. Serum levels of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are analyzed to estimate damage caused to liver. Elevated ALT, ALP & AST levels are associated with varying degrees of damage to the liver cells [3].

Homocysteine levels are also altered in chronic liver disease. Homocysteine is a sulphur-containing amino acid belonging to the group of intracellular thiols. Numerous clinical and epidemiological studies have reported that elevated plasma homocysteine concentrations reflect impaired cellular metabolism [4] and may be considered as an independent risk factor for atherosclerotic vascular disease and thromboembolism [5]. Experimental data in transgenic mice deficient in homocysteine metabolism enzymes have shown the presence of severe liver steatosis with occasional steatohepatitis. In human beings, many studies have found a correlation between homocysteine and steatosis. Hyperhomocysteinemia may result from defects in homocysteine-metabolizing genes; vitamin B₆, B₁₂, or folate deficiencies resulting from nutritional conditions; or chronic alcohol consumption [6].

Homocysteine is mainly synthesized and metabolized in the liver, since metabolism of majority of dietary methionine occurs in this organ, where about 85% of the whole body capacity for transmethylation resides. Therefore, genes involved in methionine and homocysteine metabolism are expressed in a specific pattern in the liver [7]. Homocysteine is formed as an intermediate in methionine metabolism; therefore, impaired liver function leads to altered methionine and homocysteine metabolism [8].

The gold standard therapy for chronic hepatitis C patients is a combined therapy with interferon (pegylated interferon) and an anti-viral drug (ribavirin). The first trial of interferon as therapy for chronic non-A, non-B hepatitis was reported in 1986. A sustained virologic response results from combined therapy with both pegylated interferon and ribavirin in 28%
to 50% of patients with genotype 1, while in patients with genotype 2, sustained response rates are higher (76% to 82%) [9,10]. The recommended duration of treatment for HCV genotype 2 and 3 is 24 weeks and for genotype 1 is 48 weeks. Sustained virologic response (SVR) is usually accompanied by a return to normal serum ALT levels and improvement in inflammation within the liver. Apparently, the usual effect of interferon in patients with chronic hepatitis C who respond to this therapy is viral suppression but not eradication or cure. The genotype and level of viremia are important factors that affect the initial and long-term response to this therapy. The optimal treatment for nonresponders and relapsers is not well established. However, it is expected that a minority of nonresponders (6% to 12%) will respond to a second course of pegylated interferon and ribavirin [11].

The aim of the present study was to investigate the effects of combined antiviral therapy (interferon with an antiviral drug) in patients with CLD due to HCV infection and recovery of normal serum ALT, ALP and Hcy levels in such patients.

**Subjects and Methods**

**Selection of Subjects**

Approval for the study was obtained from the Quaid-i-Azam university institutional review board. Informed consent was obtained from all subjects who participated in the study. All the patients were positive for hepatitis C infection while the control subjects were all healthy. The patients were recruited from the KRL hospital Islamabad. Patients with hepatitis B virus infection, schistosomiasis, chronic parenchymal and obstructive renal diseases and alcohol abuse were excluded from the study. 532 patients (20-68 years; 272 males, 260 females) with chronic liver disease (CLD) (Group I) and 70 healthy control subjects (18-55 years; 42 males, 28 females) (Group II) were included in the study. All the patients and controls were subjected to the following investigations: thorough history taking and physical examination, laboratory tests including complete blood cell counts (CBC), liver enzymes (ALT, ALP & AST), lipid profile (serum cholesterol, triglycerides, HDL, LDL), viral markers (PCR for HCV-RNA for those who tested positive for HCV-Ab), and plasma homocysteine concentration. Abdominal ultrasound and liver biopsy was performed for all subjects to evaluate liver and exclude any renal parenchymal disease or obstructive uropathy.

**Treatment with Interferon**

The patient group was given interferon therapy: as a first line of defence, all the patients received 3 million U of uniferon α-2B plus an antiviral drug Ribazole (1000 to1200 mg / day) for at least 24 weeks. A second line of defense therapy (Uniferon or Pegasys + Ribazole) was
given after 6 months of completion of first dose only to those patients who were either non-responders or relapsed back first line of defence.

Serum levels of HCV RNA were determined during a follow-up period (6-9 months post-treatment) with use of a reverse-transcriptase-PCR assay. All the patients were again subjected to thorough physical examination, lab tests, liver biopsy and abdominal ultrasound through and after the period of medication.

**Assessment of treatment efficacy**

A sustained virologic response, defined by the detectable levels of HCV RNA in serum 24 weeks after the end of treatment was taken as primary end point. The absence of the detectable levels of HCV RNA in serum at the end of therapy and the normalization of serum ALT levels were considered as secondary end points.

**Statistical analysis**

All values were expressed as arithmetic mean ± SD. The difference of biochemical and other parameters between the control and patient group before treatment was tested for statistical significance using t-test. For overall comparisons, one-way analysis of variance (ANOVA) was used. The difference in serum homocysteine and liver enzyme levels between the differently responding-patient groups after interferon treatment was investigated by Tukey’s honestly significant difference (HSD) test. A P-value < 0.0001 was considered extremely statistically significant, < 0.001 as very statistically significant and < 0.01 as statistically significant.

**Results**

**Hyperhomocysteinemia and serum ALT levels before interferon treatment**

532 patients (272 males; 260 females) affected with chronic liver disease (CLD) were studied. 70 healthy individuals (42 males; 28 females) were included as a control group. Hyperhomocysteinemia was defined as plasma Hcy level > 15µmol/L. The mean level of plasma Hcy was significantly higher in the patient group (25.66±8.89) when compared to control group (12.36±1.64). Hyperhomocysteinemia was observed in 486 patients (253 males; 233 females). The difference in plasma Hcy concentration reached statistical significance between the control and patient groups (table 1; figure 1). Overall ALT levels were found quite higher than the normal in the patient group (110.47±128.85). Cholesterol levels and triglyceride levels were also elevated in the patients.

**Hyperhomocysteinemia after interferon treatment**

On the basis of effect of the combined therapy given, the patient group was further divided into three groups as responders (group I: n=155; 43.66%), non-responders (group II: n=110;
30.98) and relapse-cirrhotics (group III: n=90; 25.35 %). Responders were the group of patients in which HCV RNA was found below the detection limit or turns to normal after completion of 24 weeks of interferon therapy; non-responders were those HCV patients who did not show a response to either first line or the subsequent second line of combined therapy; the third group (relapse-cirrhotics) was found normal after completion of the interferon therapy, however, retrieved back to HCV positive after 6 month of completion of therapy. The mean level of plasma Hcy was significantly reduced in responders (group-I) (19.33±2.43), when compared to group II and III (non-responders: 44.04±9.32; relapse-cirrhotics: 56.11±14.63). The difference in plasma Hcy concentration reached statistical difference among the three studied groups (table 2). At the start of the treatment, the ALT and ALP levels were higher in the three treatment groups; however, these levels fell rapidly and normalized in the responder group within 8 to 12 weeks of the first line of therapy. At the end of the 24 weeks of therapy, almost half of the patients (43.66 %; responders) had a normal ALT and ALP levels. On the contrary, the non responders did not show any decline in these levels; ALT and ALP levels declined at first in the third group, following the therapy but again became high even after treated with a second line of therapy (figure 2a,b; table 2).

**DISCUSSION**

Our results indicated that plasma homocysteine levels were significantly elevated in CLD patients in both sexes compared with control values. These findings are in accordance with the results of Taha et al who observed elevated plasma homocysteine levels in patients with liver cirrhosis secondary to hepatitis C virus (HCV) infection [4]. Garacia et al attributed this condition to the reduction of expression of main genes involved in Hcy metabolism. The degree of abnormal expression of these genes was related to the severity of liver disease [6]. Although hyperhomocysteinemia is known as atherogenic and thrombotic risk factor for cardiovascular disease, it might be also a risk factor for CLD patients; however, the direct effect of Hcy on liver injury is not well known [12]. Further more, hyperhomosysteinemia was found correlated with elevated levels of ALT, ALP, TG and cholesterol in the present study. This might be related to progression of liver injury. Several studies also have reported a correlated elevation of plasma Hcy levels with ALT, ALP, TG and cholesterol [13,14]. It is evident that homocysteine-induced ER stress leaves a dysregulated endogenous sterol response pathway, which leads to increased hepatic biosynthesis and uptake of cholesterol and triglycerides [15].

This study addressed the efficacy of a combination therapy of interferon with an antiviral drug for the treatment of CLD and recovery of Hcy, ALT and ALP levels to normal.
Interferon-α (IFN-α) is a cytokine having multiple biological functions, which includes antiviral and immunomodulatory activities [16], and is commonly used for the treatment of patients with chronic HCV infection. It has been evident from the results of several recent clinical reports [17-21] that IFN-α treatment is effective in decreasing serum ALT levels, reducing and eliminating serum HCV RNA [19-21], and improving liver histology [17-19] in patients with chronic hepatitis C. For this, a comparative analysis of sustained virologic response (SVR; clearance of serum HCV-RNA 6 months after withdrawal of therapy), homocysteinemia, serum ALT and ALP levels was carried out. Consequently, a positive response was observed in 43.66 % patients (responders), as indicated by undetectable serum HCV RNA (SVR), normal homocysteine, ALT and ALP levels; 25.35 % patients (relapse-cirrhotics) gave a positive response to therapy, however, retrieved back again to the diseased state while the therapy was found ineffective in the rest of 30.98 % patients (non-responders). Increased homocysteine levels (>16 µmol/L) represent a factor associated with a lower rate of SVR. Association between high homocysteine levels and increased oxidative stress on cells partially explains this effect [22]. It has been reported that homocysteine transulphuration through cystathionine-β-synthase (CBS) activity as well as its remethylation through betaine-dependent methyltransferase (BHMT) activity are restricted to the liver [23]. Hence, an increase in homocysteine levels could be the result of reduced activities of these enzymes in patients with liver diseases, as reported in the present study.

A 24 week course of treatment was found sufficient to prevent the chronic infection, since a second line of therapy for prolonged periods was still inefficient to reduce viremia in relapse-cirrhotics. Further more, even shorter periods of treatment might be sufficient in patients in whom serum levels of HCV RNA quickly become undetectable. These findings are in line with the results of Jaeckel et al [14]. The present report also confirmed that the current combination treatment for chronic HCV infection eliminates the virus in only about half of cases [24], therefore it is suggested that all patients with acute hepatitis C should be treated.

**CONCLUSION**

We studied effects of antiviral therapy in patients with chronic liver disease due to HCV infection. The results of the study support the importance of antiviral therapy (interferon α plus ribavirin) in these patients; and diagnostic significance of homocysteine levels in addition to other laboratory parameters.

**Competing interests**
The authors declare that they have no competing interests.
Authors' contributions
MM planned study & performed bench work. SH & MN analyzed the data and prepared the manuscript. SQ, ARK & SAM planned and supervised the research work. All authors read and approved the final manuscript.

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Relationship to serum RNA concentration, HCV-RNA genotypes, histological changes and hepatitis C virus. *J Gastroenterol Hepatol* 1996, **11**:159-165.


FIGURE LEGENDS

Figure 1: Plasma homocysteine (Hcy) levels in patients and controls.

Figure 2(a): Comparative plasma ALT levels in different patient groups at different stages of interferon therapy.

Figure 2(b): Comparative plasma ALP levels in different patient groups at different stages of interferon therapy.
<table>
<thead>
<tr>
<th>Laboratory parameters</th>
<th>Reference values</th>
<th>Control Group (n=70)</th>
<th>Patient Group (n=532)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>M:13.5-18.0; F:11.5-16.5</td>
<td>14.19±1.65</td>
<td>13.01±1.98</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>PLT (*100)</td>
<td>150-450*100</td>
<td>276.64±75.31</td>
<td>545.79±85.38</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>TLC</td>
<td>4000-11000</td>
<td>6.14±1.53</td>
<td>5.62±2.36</td>
<td>=0.01*</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>M:0-10; F:0-15</td>
<td>13.18±1.47</td>
<td>37.63±22.34</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>M:0-40; F:0-37</td>
<td>33.2±7.45</td>
<td>110.47±128.85</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>60-306</td>
<td>214.61±64.23</td>
<td>259.64±64.07</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>Normal: 3.4-5.2; Borderline high: 5.2-6.2; High: &gt;6.22</td>
<td>4.67±0.36</td>
<td>5.6±0.76</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>0.8-1.7</td>
<td>1.11±0.16</td>
<td>1.30±0.44</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>&lt;3.8</td>
<td>2.92±0.29</td>
<td>3.27±0.72</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.4-1.9</td>
<td>1.31±0.49</td>
<td>2.10±0.70</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>Hcy (µmol/L)</td>
<td>5-15</td>
<td>12.36±1.64</td>
<td>25.66±8.89</td>
<td>&lt;0.0001***</td>
</tr>
</tbody>
</table>

***: extremely statistically significant; **: very statistically significant; *: statistically significant; +: statistically non-significant. Hb: hemoglobin; PLT: platelets; TLC: total leukocyte count; ESR: erythrocyte sedimentation rate; ALT: alanine amino transferase; ALP: alkaline phosphatase; HDL: high density lipids; LDL: low density lipids; TG: triglycerides; Hcy: homocysteine
Table 2. Comparison of laboratory parameters between different patient groups after antiviral treatment

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Groups</th>
<th>Overall</th>
<th>Comparisons between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I Responders (n=155)</td>
<td>Group II Non-responders (n=110)</td>
<td>Group III Relapse-cirrhotics (n=90)</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.56±1.197</td>
<td>18.17±1.002</td>
<td>11.22±1.301</td>
</tr>
<tr>
<td>PLT (*100)</td>
<td>237.7±63.0</td>
<td>130.7±36.6</td>
<td>122.9±35.8</td>
</tr>
<tr>
<td>TLC</td>
<td>6.13±1.146</td>
<td>12.56±2.160</td>
<td>6.25±1.324</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>19.63±7.286</td>
<td>40.68±22.17</td>
<td>29.93±10.32</td>
</tr>
<tr>
<td>ALT1 (U/L)</td>
<td>118.5±36.2</td>
<td>76.07±23.9</td>
<td>102±28.8</td>
</tr>
<tr>
<td>ALT2 (U/L)</td>
<td>72.77±17.3</td>
<td>121.15±32.5</td>
<td>82.44±56.7</td>
</tr>
<tr>
<td>ALT3 (U/L)</td>
<td>41.43±9.05</td>
<td>181.32±48.4</td>
<td>115.29±24.2</td>
</tr>
<tr>
<td>ALP1 (U/L)</td>
<td>336.2±20.1</td>
<td>304.0±12.0</td>
<td>349.91±25.0</td>
</tr>
<tr>
<td>ALP2 (U/L)</td>
<td>251.6±24.0</td>
<td>340.23±10.8</td>
<td>324.87±24.0</td>
</tr>
<tr>
<td>ALP3 (U/L)</td>
<td>170.9±28.9</td>
<td>380.76±12.8</td>
<td>365.72±20.6</td>
</tr>
<tr>
<td>CHOL (mmol/L)</td>
<td>4.64±0.53</td>
<td>5.68±0.42</td>
<td>5.63±0.77</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.34±0.49</td>
<td>1.29±0.29</td>
<td>1.35±0.44</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>3.34±0.53</td>
<td>4.25±0.40</td>
<td>3.15±1.10</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.93±0.57</td>
<td>2.27±0.58</td>
<td>2.02±0.59</td>
</tr>
<tr>
<td>Hcy (µmol/L)</td>
<td>19.33±2.43</td>
<td>44.04±9.32</td>
<td>56.11±14.63</td>
</tr>
</tbody>
</table>

***: extremely statistically significant; **: very statistically significant; *: statistically significant; +: statistically non-significant. Hb: hemoglobin; PLT: platelets; TLC: total leukocyte count; ESR: erythrocyte sedimentation rate; ALT1, ALT2 & ALT3: alanine amino transferase levels before, during and after therapy, respectively; ALP1, ALP2 & ALP3: alkaline phosphatase levels before, during and after therapy, respectively; CHOL: cholesterol; HDL: high density lipids; LDL: low density lipids; TG: triglycerides; Hcy: homocysteine
Figure 1: Plasma homocysteine (Hcy) levels in patients and controls.
Figure 2(a)

Plasma ALT levels (U/L)

- Responders
- Non-responders
- Relapse cirrhotics

Legend:
- ALT1
- ALT2
- ALT3