Improved Insulin Resistance with Clearance of Chronic Hepatitis C, Genotype 4

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Abstract

Background and Aim: Greater insulin resistance (IR) is more prevalent among patients with Chronic hepatitis C virus (HCV) infection compared with those with other liver diseases and with the general population. IR affects HCV disease progression and response to treatment. The effect of HCV genotype 4 clearance on IR was prospectively evaluated.

Methods: Sixty two patients with chronic hepatitis C (CHC), genotype 4, treated with pegylated interferon-alpha-2a plus ribavirin, were classified into three groups according to response to therapy. Group I (sustained responders): 44 patients responded to treatments and had sustained virological response (SVR). Group II (non responders): 13 non responders with RNA still positive after 12 weeks therapy. Group III (relapsers): 5 who had undetectable HCV-RNA at the end of antiviral therapy but HCV-RNA relapse during follow-up (6 months after one year therapy). IR was assessed by the Homeostasis model for assessment of insulin resistance (HOMA-IR) method before and after treatment.

Results: Non of our patients were diabetic, cirrhotic, alcoholic or markedly obese. Baseline data of the three groups were comparable, with no significant statistical differences. HOMA-IR six months was decreased after treatment in the three groups, but significant only in group I.

Conclusion: In genotype 4 SVR decreases insulin resistance significantly, and such effect is not noted in non responder orrelapsers.

Key Words: Chronic hepatitis C – Genotype 4 – Sustained virological response – Insulin resistance.

Introduction

CHRONIC hepatitis C virus (HCV) Genotype 4 represents approximately 20% of global HCV infection and is the source of a considerable burden to health-care providers across the globe [1]. In Egypt, more than 90% of HCV isolates are of the G4 variant, which is significant considering Egypt has the highest worldwide prevalence of HCV (10-20%) [2].

In previous studies, HCV infection was associated with an increased risk of diabetes mellitus [3], or insulin resistance (IR) [4,5]. IR seems to be associated with liver necroinflammation [6], steatosis [6], progression of hepatic fibrosis [6], development of hepatocellular carcinoma [7], extrahepatic manifestations [8], and prognosis. Thus, IR plays a crucial role in patients with HCV infection.

In this prospective study, we evaluated the effect of HCV genotype 4 clearance on insulin resistance.

Patients and Methods

This study included 62 consecutive patients with chronic hepatitis C (CHC) between April 2004 and August 2008. The diagnosis was based on elevated serum aminotransferase level, detection of anti-HCV, and HCV-RNA and histopathological examination of liver biopsy.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and informed consent for participation in the study was obtained from each patient.

Clinical data collected before antiviral therapy included age, sex, and alcohol use. BMI was calculated as body weight in kilograms divided by the square of height in meters (kg/m²).

Venous blood sample was obtained in the morning after a 12-h overnight fast for routine investigations, blood glucose, serum aspartate
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aminotransferase, alanine aminotransferase, γ-glutamyltransferase, albumin and total bilirubin. Fasting immunoreactive insulin (IRI) levels was measured by ELISA method (Mercodia ELISA, Sweden).

All CHC patients were defined by the presence of anti-HCV antibodies and detectable serum HCV RNA (HCV RNA quantitative: Cobas Amplicor HCV test Roche Diagnostics). HCV genotyping was performed (INNO-LIPA) in all patients [9]. High viral load was defined as serum HCV RNA level >600,000IU/mL.

Liver biopsy and histopathological examination was performed before starting treatment for all patients.

IR was assessed by the homeostasis model for assessment of insulin resistance (HOMA-IR) method [10], using the following equation:

\[
\text{HOMA-IR} = \frac{\text{fasting insulin (µU/mL)} \times \text{fasting glucose (mmol/L)}}{22.5}
\]

IR was calculated before starting therapy and 6 months after end of treatment for all patients. A HOMA-IR index value of more than 2.0 was considered as the criterion of insulin resistance, and that higher than 4.0 as a prediabetic state [12].

Patients with the following conditions were excluded: Well established cirrhosis, positive HBsAg (for CHC patients), human immunodeficiency virus infection, autoimmune hepatitis, hemochromatosis, α1-antitrypsin deficiency, Wilson’s disease, diabetes mellitus, corticosteroid therapy, pancreatitis or pancreatic tumors, and obesity with BMI above 30.

Histological data:

For each patient, a liver biopsy specimen was taken and fixed in 10% formalin buffer and stained with hematoxylin-eosin. Liver biopsy specimens were evaluated by a pathologist who was unaware of the patients’ clinical and laboratory data. The specimens were scored according to the METAVIR scoring system [11], which is suited for evaluation of CHC.

Treatment Outcome:

All patients in this study were treated with pegylated interferon-alpha-2a 180 µg injected subcutaneously once weekly plus ribavirin 1200mg daily, with dose alteration if needed.

Patient population was classified into three groups according to their response to therapy.

- **Group I (sustained responders):** Included 44 patients responded to treatments and had sustained virological response (SVR) with negative RNA 6 months after one year therapy.
- **Group II (Non-responders):** Included 13 patients who showed no response with RNA still positive after 12 weeks therapy.
- **Group III (relapers):** Included 5 patients who had undetectable HCV-RNA at the end of antiviral therapy but HCV-RNA relapse during follow-up 6 months after one year therapy.

Statistical analyses:

Continuous variables were summarized as mean (±SD) and categorical variables as frequency and percentage. Comparisons between groups were performed using the Student t test. Correlations between pairs of numerical variables were performed using the Person rank correlation method. All p values were based on a 2-sided test of statistical significance. Significance was accepted at p<.05. All analyses were performed with SPSS software for windows, version 12.0 (SPSS Inc, Chicago, IL).

Results

This study included 62 patients, 50 males and 12 females, all patients were Egyptian, having genotype 4. All patients in this study were treated with pegylated interferon 180 µg injected subcutaneously once weekly plus ribavirin 1200mg daily, with dose alteration if needed.

Non of the included patients were diabetic, cirrhotic, alcoholic or markedly obese, although 18 (29%) were overweight with BMI above 25, of these 12 (27%) in group I, 4 (33%) in group II and 2 (40%) in group III.

Characteristics of the three patients groups presented in Table (2), non significant increase in mean age, BMI, viral load, necroinflammation, fibrosis, and HOMA-IR was noted in group II,
III in comparison with group I. Meanwhile, non significant decrease in BMI was noted in the three groups after treatment. HOMA-IR 6 months after treatment in the three groups was decreased, but significant only in group I.

Table (1): Characteristics of the whole patients involved in the study.

<table>
<thead>
<tr>
<th></th>
<th>Sustained responder</th>
<th>Non- responder</th>
<th>Relapser</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>44</td>
<td>13</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>42.91±6.95</td>
<td>45.08±5.47</td>
<td>46.40±7.44</td>
<td>0.381</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>36/8</td>
<td>10/3</td>
<td>4/1</td>
<td></td>
</tr>
<tr>
<td>BMI before treatment</td>
<td>23.70±3.43</td>
<td>23.82±2.65</td>
<td>24.66±3.38</td>
<td>0.826</td>
</tr>
<tr>
<td>BMI after treatment</td>
<td>23.54±3.15</td>
<td>23.52±2.58</td>
<td>24.42±2.95</td>
<td>0.824</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>139.39±51.85</td>
<td>140.08±60.69</td>
<td>101.80±21.56</td>
<td>0.310</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>98.89±39.98</td>
<td>109.92±51.85</td>
<td>75.80±15.80</td>
<td>0.301</td>
</tr>
<tr>
<td>γ-GT (U/L)</td>
<td>89.02±70.39</td>
<td>80.00±42.90</td>
<td>61.60±35.52</td>
<td>0.690</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>0.68±0.31</td>
<td>0.69±0.39</td>
<td>0.63±0.32</td>
<td>0.917</td>
</tr>
<tr>
<td>Albumin</td>
<td>3.52±0.44</td>
<td>3.46±0.31</td>
<td>3.46±0.28</td>
<td>0.862</td>
</tr>
<tr>
<td>Viral load</td>
<td>516.06±434.21</td>
<td>672.92±488.89</td>
<td>752.20±314.49</td>
<td>0.333</td>
</tr>
<tr>
<td>Necroinflammation</td>
<td>2.84±0.75</td>
<td>3.2±0.83</td>
<td>3.2±0.83</td>
<td>0.520</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>2.16±0.96</td>
<td>2.38±0.65</td>
<td>2.6±1.14</td>
<td>0.496</td>
</tr>
<tr>
<td>HOMA-IR before treatment</td>
<td>3.76±1.85</td>
<td>4.00±2.29</td>
<td>4.08±2.24</td>
<td>0.089</td>
</tr>
<tr>
<td>HOMA-IR after treatment</td>
<td>3.40±1.52</td>
<td>3.81±1.74</td>
<td>4.02±2.07</td>
<td>0.569</td>
</tr>
</tbody>
</table>

Non significant p value for comparison among the three groups in all parameters.

Table (2): Characteristics of the three groups involved in the study.

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</tr>
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<td>Sex (M/F)</td>
<td>50/12</td>
<td>10/3</td>
<td>4/1</td>
<td></td>
</tr>
<tr>
<td>BMI before treatment</td>
<td>23.80±3.23</td>
<td>23.61±2.99</td>
<td>24.66±3.38</td>
<td>0.826</td>
</tr>
<tr>
<td>BMI after treatment</td>
<td>99.34±41.73</td>
<td>99.34±41.73</td>
<td>99.34±41.73</td>
<td>0.569</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>84.92±70.35</td>
<td>84.92±70.35</td>
<td>84.92±70.35</td>
<td>0.569</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.68±0.32</td>
<td>0.68±0.32</td>
<td>0.68±0.32</td>
<td>0.569</td>
</tr>
<tr>
<td>Albumin (gm/dL)</td>
<td>3.50±0.4</td>
<td>3.50±0.4</td>
<td>3.50±0.4</td>
<td>0.569</td>
</tr>
<tr>
<td>Viral load</td>
<td>568±539.86</td>
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</tr>
<tr>
<td>Fibrosis</td>
<td>2.24±0.92</td>
<td>2.24±0.92</td>
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<td>HOMA-IR before treatment</td>
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</tr>
<tr>
<td>HOMA-IR after treatment</td>
<td>3.54±1.6</td>
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</tr>
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</table>

Non significant p value for comparison among the three groups in all parameters.

Discussion

In a Spanish study 525 CHC patients treated with peginterferon plus ribavirin were followed-up after treatment. The incidence of altered baseline glucose and the appearance of diabetes type 2 was greater in non-responders than in sustained responders. In their conclusion, HCV clearance induces a

Also, HOMA-IR correlates with BMI, viral load, and fibrosis grade in liver biopsy.

In a Spanish study 525 CHC patients treated with peginterferon plus ribavirin were followed-up after treatment. The incidence of altered baseline glucose and the appearance of diabetes type 2 was greater in non-responders than in sustained responders. In their conclusion, HCV clearance induces a
decrease in insulin resistance index during a short time follow-up and decrease the incidence of diabetes type 2 in long-term follow-up [12].

Besides, in 52 patients from UK treated with peginterferon plus ribavirin, HOMA index was significantly higher in non-responders than patients with sustained response [13].

Also, supporting this hypothesis, in a study evaluating the treatment of 89 patients with CHC showed that eradication of HCV improved IR, cell function, and hepatic insulin receptor substrates (IRS-1/-2) expression [14]. Nonresponders and relapers experienced no significant changes in HOMA-IR or HOMA for measuring cell function values after treatment with INF-alfa-based therapy, however, HOMA-IR and HOMA- 0 values significantly decreased in sustained responders. Furthermore, the change in IR was accompanied by a 2-fold increase in hepatocyte expression of IRS-1 and a 3-fold increase in IRS-2 levels, suggesting a direct interaction between HCV and IRS -1/-2 in the development of IR.

Moucari, et al. [15] implicated infection with viral genotypes 1 or 4 as an independent contributor to IR. They found that, IR was more frequently present in HCV than in general French population. These data suggest that IR represents not only a direct viral feature. Several mechanisms have been proposed to explain HCV-induced IR [18,19], and all suggest direct impairment of the insulinsignaling pathway [20,21].

Several studies reported that baseline IR had a negative impact on treatment outcomes in patients with CHC [22-24]. Romero-Gomez, et al. [25] reported a direct correlation between IR and SVR. Furthermore, HOMA-IR scores did not change in patients who did not respond to treatment, whereas HOMA-IR scores decreased progressively during treatment in patients who attained SVR. Interestingly, inrelapers, HOMA-IR scores decreased during treatment, but returned to baseline values during follow-up evaluation, supporting a virologic basis for IR in these patients.

Simo, et al. [26] showed that significantly more patients with normal fasting glucose levels and biopsy-proven CHC who did not attain SVR developed DM during follow-up evaluation compared with patients who attained SVR after treatment with IFN-alpha-2b with or without RBV.

In this work, IR correlates with degree of fibrosis. The main deleterious effect of IR in CHC is the ability to promote fibrosis progression. High serum glucose levels have been associated with an increased rate of fibrosis progression, greater even than overweight [27]. Mean HOMA index increases with the stage of fibrosis [28] and could help to differentiate stages of fibrosis. Moreover, Sud, et al [29] proposed an index to predict fibrosis containing age, cholesterol, $y$-GT and alcohol consumption together with HOMA.

In our study, BMI values were within normal limits in 71% or overweight in 29% while obese patients were excluded. Improved HOMA-IR was only seen in sustained responders and HOMA-IR remained unchanged in nonresponders, despite a decrease in BMI after antiviral therapy for all patients group.

Although, obesity is a common factor for the development of IR [30], greater IR than could be explained by obesity or overweight, was seen in patients with CHC. In an epidemiologic study, Bahtiyar, et al. reported that obesity is not associated with the development of IR in patients with HCV infection [31]. In addition, the development of IR is seen by 1 month of age, in the absence of either overt liver injury or excessive body weight gain in HCV core transgenic mice [32] and serum HCV core protein levels are associated with HOMA-IR values in patients with CHC [33]. Taken together, these findings suggest that HCV itself causes IR.

Moreover, in this study IR correlates with HCV RNA levels. In fact, in the absence of obesity and significant fibrosis, HOMA-IR correlated significantly with serum HCV RNA levels, suggesting a direct role of viral replication on the development of IR. Several studies reported a positive correlation between viral load and IR [34,35]. Kawaguchi et al. [36] reported that patients with CHC and high baseline viral load had significantly increased fasting serum insulin levels and HOMA-IR scores compared with patients with low viral load.

In conclusion, HCV promotes insulin resistance that interferes with response to treatment, in genotype 4 SVR decreases insulin resistance significantly, and such effect is not noted in non responder or relapers.

References


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