Impact of insulin resistance on early virological response in non-diabetic, non-cirrhotic HCV patients treated with peginterferon alpha-2b plus ribavirin and correlation with biologic, Hematologic and biochemical parameters

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Abstract: Background: The therapy of hepatitis C virus (HCV) has improved in recent years and eradication of HCV by treatment is a reality in many chronically infected patients; many host and viral factors influence the virological response rate to combined interferon and ribavirin therapy. Viral factors include viral load and genotype, whereas host factors include age, gender, body mass index (BMI) and insulin resistance (IR). Objective: We aimed to assess the impact of IR on early virological response (EVR) in HCV patients treated with peginterferon alpha-2b plus ribavirin. Also, we assess other pretreatment variables might predict an EVR. Material and Methods: The study was conducted on 60 patients with chronic active hepatitis C fulfilling the criteria for treatment with interferon and ribavirin, insulin resistance index using HOMA-IR was measured before starting therapy, HCV antibody and HCV-RNA by quantitative PCR were performed. Follow-up with quantitative HCV-RNA was done after 12 weeks for EVR. Results: Of 60 patients, 13 patients were non-responders (21.7%) and 47 patients showed EVR (78.3%). Pretreatment HCV RNA level, HOMA-IR and age were independent predictors for EVR. High HOMA-IR is associated with poor response. Also, there was statistically significant relation between BMI and EVR (P<0.05). Regarding the age, there was a significant statistical difference between responders and non responders. No statistically significant difference between EVR and CBC parameters. Conclusion: HOMA-IR appears to be a useful tool in predicting EVR and should be evaluated at baseline in non-diabetic, non-cirrhotic HCV patients before initiating antiviral treatment while CBC parameters have no effect on EVR.


Keyword: Insulin resistance - HOMA-IR- Early virologic response - Chronic hepatitis C

Abbreviations: RVR, rapid virologic response; SVR, sustained virologic response; HOMA-IR, homeostasis model assessment–insulin resistance; BMI, body mass index; HCV, hepatitis C virus; EVR, early virological response; RBV, ribavirin

1. Introduction

The prevalence of chronic hepatitis C is relatively high in the Middle East, especially in Egypt, the predominant type is genotype 4 [1]. The therapy of hepatitis C virus (HCV) has improved in recent years and eradication of HCV by treatment is a reality in many chronically infected patients, but the issue of a substantial number of non responders is still unsolved and the introduction of pegylated interferon in combination with ribavirin has greatly improved the treatment outcome of HCV infection [2].

Many host and viral factors influence the sustained virological response rate to combine interferon and ribavirin therapy. The viral factors include viral load and genotype [3] whereas host factors include age, gender, host immune status, HLA, [4] ethnicity and body mass index (BMI) [5].

After initiation of treatment, initial viral decline with undetectable HCV-RNA at week 4 of therapy is the best predictor of sustained virologic response (SVR) independent of HCV genotype [6] while higher Insulin resistance (IR) is associated with poor response to antiviral treatment [4].

The relationship between antiviral treatment and IR in patients with chronic hepatitis C has not yet been completely elucidated. It is known that hyperinsulinemia is associated with increased HCV
replication in genotype 1 patient [7]. IR is involved in fibrogenesis and is associated with more severe fibrosis in genotype 1 patients [8].

In the current study, we aimed to assess the impact of IR and hematologic parameters (CBC) on early EVR in HCV patients treated with pegylated interferon alpha-2b plus ribavirin. Also, we assess other pretreatment variables might predict an EVR.

2. Patients and Methods
Patient Selection:
This study included sixty patients diagnosed as chronic active hepatitis C recruited from the Hepatology Clinic of El Obour Hospital, Kafr El Sheikh and Benha University Hospital, Egypt from January 2012 to February 2013. The enrolled patients were fulfilling the criteria for treatment with interferon and ribavirin. They were 45 males (75%) and 15 females (25%). Their ages ranged from 18-55 years at the start of therapy. We obtained institutional ethics board approval for this study.

Inclusion Criteria were: age between 18-60 years, elevation of serum transaminase (AST and ALT) levels for more than 6 months duration, positive serum HCV-RNA by PCR, HCV-RNA level greater than 1000 IU/ML, creatinine level less than 1.5 mg/dl, evidence of chronic hepatitis in liver biopsy and patients not previously treated with any antiviral drugs.

The exclusion Criteria include younger than 18 years or older than 60 years, liver cirrhosis, diabetes mellitus, hepatocellular carcinoma, hepatitis B virus infection, HIV infection, alcoholics, autoimmune disorders, thyroid dysfunction, pregnant and breast feeding women were excluded from the study.

All patients were subject to the following:
1. Full history taking and clinical examination with special stress on abdominal examination, signs of liver cell failure and fundus examination.
2. Insulin resistance index using HOMA-IR (Homeostasis Model of Assessment Insulin Resistance Index). HOMA-IR was calculated on the basis of fasting values of plasma glucose and insulin according to the HOMA model formula: HOMA – IR [fasting insulin (mIU/L) x fasting glucose (mmol/L)] / 22.5. For statistical purposes, HOMA – IR was expressed as a categorical variable (<2 vs ≥2) [4, 9].
3. BMI: Height and weight were determined and BMI calculated as weight in kilograms divided by height in meters squared. Patients with a BMI between 25 and 29.9 kg/m² were categorized as overweight, and patients with a BMI ≥ 30 kg/m² categorized as obese [10].
4. Laboratory investigations including complete liver functions (serum levels of AST, ALT, ALP, GGT, total bilirubin, direct bilirubin, PT, total protein and albumin), complete blood picture, renal functions (serum Creatinine and blood urea), fasting blood sugar, hepatitis B surface antigen (HBsAg), alpha fetoprotein, pregnancy test (qualitative β-HCG in serum), antinuclear antibodies (ANA), Thyroid-stimulating hormone (TSH).
5. Test for bilharzial antibodies by indirect hemagglutination test. Those who showed high antibody titer (>1/160) received antibilharzial drugs in the form of praziquantel in a dose of 40 mg/kg as a single oral dose.
6. HCV antibody using ELISA technique and HCV-RNA by PCR quantitative (before starting therapy).
7. Abdominal ultrasonography.
8. Liver biopsy with histopathological scoring (grading and staging)
9. All the patients were treated with pegylated interferon-2b at a dose of 1.5 µg/kg body weight-t subcutaneously every week plus ribavirin at a dose of 15 mg/kg/day orally.
10. Quantitative PCR for HCV-RNA after 12 weeks was done to assess EVR.
11. Fasting plasma insulin (before starting therapy).

Follow-Up
All patients were followed up regarding clinical and biochemical parameters every week for 1 month, every 2 weeks during therapy. CBC and aminotransferase levels were measured at weeks 1, 2 and 4 and at 4 weeks interval thereafter.

The dose of ribavirin was adjusted downwards (by 50%) if significant anemia occurred (hemoglobin less than 10 g/dl or decrease in hemoglobin by 2 g/dl during any 4-week period treatment) and ribavirin was stopped if severe anemia occurred (hemoglobin <8.5 g/dl). The dose of peg-interferon alfa-2b was adjusted downward if intolerable side effects occurred such as severe fatigue, depression, or irritability of marked decrease in white blood cell counts (absolute neutrophil count below 750 cells / mm³). The dose of peg-interferon alfa-2b was reduced from 1.5 to 1.0 and then to 0.5 mcg per kg per week. Peg-interferon was stopped if marked decrease in white blood cell counts (absolute neutrophil count below 500 cells / mm³) or platelet counts (below 50,000 cells / mm³). These were according to product information guidelines for dose modification and discontinuation of PEG-IFN alfa-2b/PEG-IFN alfa-2b plus ribavirin for hematologic toxicity.

At 12 weeks, HCV-RNA levels were measured. The therapy was stopped if HCV-RNA levels at week 12 had not decreased by at least two log 10 units. For those with negative HCV-RNA by PCR at week 12
or showed a decrease by more than two log 10, therapy was continued.

**Statistical Analysis:**

The data were coded, entered and processed and analyzed using SPSS statistical software package. Data were expressed as Mean and standard deviation (SD) for quantitative measures and both number and percentage for categorized data using Student’s t-test to compare two independent mean groups for parametric data, Chi-square test for comparison of qualitative variables with each other and Z-test Comparison between 2 portions. The probability of error (P) was expressed P-Value ≤ 0.05 is significant and P-Value < 0.01 is highly significant.

**3. Results**

The patient’s characteristics and laboratory data are summarized in table 1. Of 60 patients diagnosed as having chronic active hepatitis C, 45 were males (75 %) and 15 females (25 %), the mean age was 38.56 years (range, 18-55). The mean body mass index was 26.24 Kg/m², (range, 19 - 33 kg/m²) and the patients were classified according BMI into patients with BMI< 30 kg/m² (41 cases) and patients with BMI: > 30 kg/m² (19 cases). IR by HOMA scored higher than 2, was 30 % of patients and 70% scored less than 2 (HOMA-IR mean was 2.15).

The mean viral load was 490,539 IU/ml (range, 5,043- 9,556,800 IU/ml), 46 patients (76.67%) had low and moderate viraemia (PCR < 4000000 IU/ml) while 14 patients (23.33%) had high viremia (PCR > 400000 IU/ml), there was statistically highly significant difference in response to treatment regarding pretreatment viral load (P<0.01). Early virological response was 78.33 % (47 patients) while 21.67% (13 patients) failed to achieve response. Regarding the age with virological response at 12 week, there was a significant statistical difference (P < 0.05) between responders and non responders, non responders had a higher age (mean 41.1 ± 4.5) than responders (mean 37.7± 7.5), while, there was no significant statistical difference between males and females regarding response at week 12. There was highly significant difference between EVR and non responders as regarding HOMA-IR, Mean HOMA-IR were less in responder than non responders (figure 1). Also, there was statistically high significant relation between BMI and EVR (P < 0.05). A positive correlation was found between BMI and HOMA-IR. HOMA-IR increased with increasing BMI, and was statistically significant (P <0.05). No statistically significant difference between EVR and CBC parameters (p > 0.05) as shown in table 2.

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Early virological response (n# 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Responder (N=47)</td>
</tr>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td><strong>Gender:</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>45</td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
</tr>
<tr>
<td><strong>Age group:</strong></td>
<td></td>
</tr>
<tr>
<td>&gt; 40years</td>
<td>21</td>
</tr>
<tr>
<td>&gt; 40years</td>
<td>39</td>
</tr>
<tr>
<td><strong>BMI (kg/m²):</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 30</td>
<td>41</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>19</td>
</tr>
<tr>
<td><strong>HOMA-IR</strong></td>
<td></td>
</tr>
<tr>
<td>&gt; 2</td>
<td>18</td>
</tr>
<tr>
<td>&lt; 2</td>
<td>42</td>
</tr>
<tr>
<td><strong>ALT</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 3 fold increase(120)</td>
<td>40</td>
</tr>
<tr>
<td>&gt; 3 fold increase(120)</td>
<td>20</td>
</tr>
<tr>
<td><strong>Alkaline phosphatase:</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; ULN(175)</td>
<td>42</td>
</tr>
<tr>
<td>&gt; ULN(175)</td>
<td>18</td>
</tr>
<tr>
<td><strong>PCR (IU/ml):</strong></td>
<td></td>
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<tr>
<td>&gt; 400000</td>
<td>14</td>
</tr>
<tr>
<td>&lt; 400000</td>
<td>46</td>
</tr>
</tbody>
</table>
Table (2): Relation between EVR and CBC parameters.

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Early virological response</th>
<th>No.</th>
<th>Mean ± SD</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBCs</td>
<td>Responder</td>
<td>47</td>
<td>5,742.6 ± 1259.7</td>
<td>0.1</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Non responder</td>
<td>13</td>
<td>5,784.6 ± 1434</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Responder</td>
<td>47</td>
<td>13.9 ± 0.9</td>
<td>1.55</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Non responder</td>
<td>13</td>
<td>13.5 ± 0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>Responder</td>
<td>47</td>
<td>165,659.6 ± 34,678</td>
<td>0.22</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Non responder</td>
<td>13</td>
<td>168,615 ± 44,331</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure (1): Relation between HOMA-IR and early Virological response

4. Discussion:

The effectiveness of therapy for chronic hepatitis C patients has greatly improved in the last few years. The current standard of care is a combination of pegylated interferon-alpha and ribavirin [6]. IR, glucose intolerance and diabetes mellitus are commonly associated with cirrhosis. The exact pathogenetic mechanisms responsible are still unknown; however, they may be related to both hepatitis C virus itself and to liver injury [11].

In the current study, responders were 47 out of 60 patients (78.3%) while 13 patients (21.7%) failed to achieve response; there was statistically significant correlation between age and EVR, non responders had a higher age (mean 41.1 ± 4.5) than responders (mean 37.7± 7.5) and EVR was a predictor of SVR with subsequent statistically significant correlation between age and SVR. These results are similar with the results of previously published studies [2, 3, 12, 13], which reported that younger age correlated significantly with an SVR and patients younger than 40 years showed the best response rates. In contrast to our results, few studies [1, 14] mismatch with our results, they reported that there was no impact of age (<40 years, >40 years) on virological response in 95 and 66 Egyptian patients infected with HCV genotype 4 treated with PEG-IFN-α 2b plus ribavirin respectively, this difference may be attributed to small size sample.

In the present study, there was a higher percentage of responders at 12 week among cases with sensitive HOMA-IR compared to resistant cases, mean HOMA - IR was less in responder than non responders and was statistically highly significant (P <0.01), this findings agreed with the result by other [4] who found that HOMA - IR was lower in patients with better virological response. Also our results agree with Zhu et al., [15] who found insulin resistance to be an independent prediction factor for non response to treatment in chronic hepatitis C patients. Also, in another study carried on 159 patients with chronic HCV, insulin resistance index was found to be an independent predictor of response to antiviral therapy in chronic HCV patients treated with PEG IFN plus ribavirin [4]. Our results go with that previously published [16] who reported that non responders to antiviral therapy had significantly higher insulin level. On the other hand, our results disagree with other study, [17] which revealed that IR is frequently encountered in HCV-genotype 4 patients, and IR is not a negative predictor of response to treatment.

In the present study a positive correlation was found between mean BMI and HOMA-IR. HOMA-IR increased with increasing BMI and was statistically significant (P <0.05). This agrees with others [4, 18, 19], who found that IR was correlated with BMI.

In the current study, Patients with BMI <30
kg/m is associated with better response rates (76.60% of responders) whereas patients with high BMI >30 kg/m (obese patients) represented 23.40% of responders with statistically high significant correlation between BMI and response to treatment. Our results agree with others [2, 3, 4, 5, 20, 21] who found that BMI was lower in responders compared to non-responders. However, our results disagree with others [22, 23] who didn’t confirm correlation between BMI and response to treatment through the multilogistic regression analysis including BMI and body weight, also, other investigators [14] found that there was no impact of weight or BMI on response to treatment.

In the current study, there was no statistically significant difference between EVR and CBC parameters (p > 0.05), a similar finding was reported by [13]. However, the role of anemia upon EVR and SVR is controversial, we didn’t see evidence of a difference in EVR or SVR among those with and without anemia (P> 0.05), although the rates of both EVR and SVR were slightly higher among patients who didn’t experience anemia. However, this is in contrast to two recent studies which reported higher rate of SVR among those who developed anemia and significantly more likely to achieve response to HCV treatment [24, 25], the reason for this remains unclear.

Similarly, our results revealed no statistically significant difference between gender and EVR, these findings with similar to previously reported by others [4, 14]. There was statistically high significant difference in response to treatment regarding baseline viral load, we found that the viral load determined before treatment was significantly lower in responder than in non-responder patients (P<0.01). This finding is similar to previously reported [23, 26, 27, 28] who found that assessment of viral load before therapy is an important tool for the prediction of treatment outcome and that a low pretreatment viral load was a predictor of virological response regardless of genotype. On the other hand, in a study done by other, [14] found no impact of viral load (<600,000, >600,000 IU/ml) on virological response.

In this study, there was no statistically significant difference in any of liver profile tests (ALP & ALT) between patients with absent or present EVR (p > 0.05), this findings was reported by others [13,24,29] who founded that there was no statistically significant difference in any of liver profile tests between patients with absent or present virological response. On the other hand, Foster et al., [30] found positive correlation between response to therapy and an ALT level above 3 times.

Summary

In conclusion, the baseline factors found to be predictive for the presence of EVR were BMI <30 kg/m, HOMA-IR< 2, low and moderate viraemia (PCR< 40000 IU/ml), and age < 40 years. Large study even national research study is required to confirm these important findings which affect patient’s response and outcome

Footnotes

Source of support: Nil

Conflict of Interests: The authors declare that they have no competing interests.

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