Evaluation of Interleukin-8 and HCV RNA in Chronic Hepatitis C Patients as Predictors of Response to Pegylated Interferon/Ribavirin Therapy at 12 and 24 Weeks

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Abstract

Background: Numerous studies suggest that HCV-induced changes in levels of chemokine and cytokine expression may be involved in HCV antiviral resistance, persistence, and pathogenesis. Also found that the HCV NS5A protein induces expression of the proinflammatory chemokine IL-8 to partially inhibit the antiviral actions of IFN in vitro.

Aim: To investigate the impact of serum levels of interleukin-8 in patients with chronic hepatitis C viral infection and baseline HCV RNA levels as predictors for response to Interferon/Ribavirin therapy in patients with chronic HCV infection in the centre for treatment of HCV in Ismailia Fever Hospital-East Egypt.

Methods: This descriptive study was conducted on 42 patients to determine the correlation between levels of interleukin-8 in the serum of patients with chronic hepatitis C viral infection and baseline HCV RNA and response to pegylated Interferon/Ribavirin after 12 and 24 weeks of therapy. All investigations and follow-up had been carried out in the centre according to the national program for treatment of chronic hepatitis C virus.

Results: Among the total studied 42 patients the mean age was 41.5 years old. About half of the studied patients were males (52.4%) and more than half of the patients were from rural areas (61.9%).

A 73.8% of the studied patients were responders to interferon therapy while 26.2% were non responders. Seven (16.7%) of these non responders have shown positive PCR and discontinue treatment at 12 weeks while the remaining 4 patients (9.5%) were recorded as non responders at 24 weeks of therapy. There was no statistically significant difference between responders and non responders regarding age, sex and residence. Responders have significantly lower hemoglobin values, FBS, a-fetoprotein and baseline PCR than non responders. More than half of the whole studied patients have IL-8 value 2-6 (mid range elevation) (64.3%) while 35.7% shows high level of IL-8 (>14). Responders have lower mean of IL-8 with statistically significant difference than non responders (7.9 versus 13.3). Most of responders (74.2%) have IL-8 value ranging from 2-6 while most of non responders (63.6%) have high level of IL-8 (>14). Patients with IL-8 (2-6) are less likely to be non responder with RR=0.3 (95% CI: 0.1-0.9) while patients with IL-8 >14 are at higher risk of being non responder with RR=3.1 (95% CI: 1.09-9.03).

Conclusion: Most of responders have low levels of pretreatment IL-8, while most of non responders have high levels of IL-8, and also Low pretreatment levels of HCV RNA were predictive to response.

Key Words: HCV – RNA – Chronic hepatitis C – Pegylated interferon/ribavirin therapy.

Introduction

HEPATITIS C virus (HCV) infection is a major cause of liver diseases and liver cancer [1-3]. Among the six genotypes of HCV, the most common genotypes of HCV in the United States are genotype 1 (approximately 75%), genotype 2 (15%), and genotype 3 (7%) [2-4]. Egypt has the largest epidemic of hepatitis C virus (HCV) in the world. The recently released Egyptian Demographic Health Survey tested a representative sample of the entire country for HCV antibody. The sample included both urban and rural populations and included all governorates of Egypt. Over 11,000 individuals were tested. The overall prevalence (percentage of people) positive for antibody to HCV was 14.7% [8].
Among the six major HCV genotypes found worldwide, genotype 4 is the most predominant in Egypt, with 4a as the dominant subtype [6].

At present, the standard treatment for chronic HCV genotype 4 infections is 48 weeks with a combination of pegylated interferon alfa-2a or alfa-2b plus ribavirin. Outcomes are measured by sustained viral response (SVR), defined as an undetectable viral load 24 weeks after the end of therapy. HCV genotype 1 has been reported to have a 54-56% SVR [2,7-11]. Prior studies have shown better response rates with genotype 2 or 3 with a 24 week-course of therapy [2,10,11]. Response rates have been found higher in Caucasians (52%) compared to African-Americans (28%) [2]. Response rates are reported lower with initial levels of HCV RNA>600,000IU/ml, male gender, high body weight, and advanced liver fibrosis [12-18], also shown a poorer response to treatment associated independently with HCV genotype 1 infection but a better response with weight Loss [19].

Numerous studies suggest that HCV-induced changes in levels of chemokine and cytokine expression may be involved in HCV antiviral resistance, persistence, and pathogenesis. Also found that the HCV NS5A protein induces expression of the proinflammatory chemokine IL-8 to partially inhibit the antiviral actions of IFN in vitro [20].

NS5A induction of IL-8 expression is associated with inhibition of the antiviral actions of IFN in vitro [21]. This may represent a distinct mechanism by which the NS5A protein circumvents the IFN-induced antiviral response. It was also demonstrated that the HCV core protein could also transactivate the IL-8 promoter [22].

Interferon (IFN) and the guanosine analogue ribavirin are widely used treatments for chronic HCV infection. However, 60% of patients with high-titer HCV genotype 1 infections remain non-responsive to combination therapy [20].

The high prevalence of hepatitis C, and the need to understand its epidemiology, warrants global surveillance of the disease in order to determine specific health care measures for disease prevention and control. Several studies have been carried out to identify factors that facilitate identification of chronic hepatitis C patients who are likely to respond to antiviral treatment. So in this study we investigated pretreatment levels of both IL-8 and HCV RNA as predictors for response to interferon/ribavirin therapy in patients with chronic HCV infection.

Material and Methods

Study population:
This prospective descriptive study enrolled 42 patients with chronic HCV infection suitable for interferon/ribavirin therapy, both genders and all age groups above 18 years old attending to the center for treatment of chronic HCV in Ismailia Fever Hospital, Egypt from May 2011 to February 2012.

Data collection:
The clinical data was collected in a pre-organized data sheet which included sociodemographic data regarding age, sex, residence, telephone number and medical history.

Clinical assessment, included, general and regional examination (heart, chest and abdominal examination) and fundus examination.

Investigations included laboratory investigations: Complete blood count, fasting blood sugar, S creatinine, S albumin, alkaline phosphatase, aspartate aminotransferae (AST), alanine aminotransferase (ALT), S bilirubin, prothrombin time (INR), HbsAg, anti-HCV Ab, HCV RNA by PCR, Thyroid Stimulating Hormone (TSH), antinuclear antibodies (ANA), alpha-fetoprotein (AFP), pregnancy test for females, urine analysis and liver biopsy. Serum level of interleukin-8 was assessed by ELISA. Follow-up to assess response to interferon therapy within either 12 or 24 weeks of treatment by PCR.

Results
Among the total studied 42 patients a 73.8% of the studied patients were responders while 26.2% were non responders (Table 1).

<table>
<thead>
<tr>
<th>Table (1): Distribution of the studied patients according to response to interferon therapy (n=42).</th>
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<tbody>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Responders</td>
</tr>
<tr>
<td>Non responders</td>
</tr>
<tr>
<td>At 12w</td>
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<tr>
<td>At 24w</td>
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</table>

More than half of the whole studied patients have IL-8 value 2-6 (mid range elevation) (64.3%) while 35.7% shows high level of IL-8 (≥ 14). (Table 2).
Table (2): IL-8 level among the studied patients (n=42).

<table>
<thead>
<tr>
<th>Number</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>Mean±SD</td>
<td>9.4±6.9</td>
</tr>
<tr>
<td>Range</td>
<td>2.3-26</td>
</tr>
<tr>
<td>IL-8</td>
<td></td>
</tr>
<tr>
<td>Mid range (2-6)</td>
<td>27</td>
</tr>
<tr>
<td>High level (≥14)</td>
<td>15</td>
</tr>
</tbody>
</table>

Responders have lower mean of IL-8 with statistically significant difference than non responders (7.9 versus 13.3). Most of responders (74.2%) have IL-8 value ranging from 2-6 while most of non responders (63.6%) have high level of IL-8 (≥14). Patients with IL-8 (2-6) are less likely to be non responder with RR=0.3, while patients with IL-8 ≥14 are at higher risk of being non responder with RR=3.1 (Table 3).

Table (3): Comparison between responders and non responders regarding IL-8 value.

<table>
<thead>
<tr>
<th></th>
<th>Non responder (n=11)</th>
<th>Responder (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>13.3±7.6</td>
<td>7.9±6.2</td>
</tr>
<tr>
<td>Range</td>
<td>3.7-24</td>
<td>2.3-26</td>
</tr>
<tr>
<td>Mid range</td>
<td>4 (36.4%)</td>
<td>23 (74.2%)</td>
</tr>
<tr>
<td>High level</td>
<td>7 (63.6%)</td>
<td>8 (25.8%)</td>
</tr>
</tbody>
</table>

*Statistically significant difference (p-value <0.05).

From our results we noticed that there is a statistical significant difference between responders and non-responders regarding baseline HCV RNA by PCR (x10^7) as the mean for responders was 5.9±10.7 and for non-responders was 36.8±60.4 with p-value (0.001).

Table (4): Difference of IL-8 between responders, non responders and breakthrough.

<table>
<thead>
<tr>
<th></th>
<th>Responders (n=31)</th>
<th>Non-responders (n=7)</th>
<th>Breakthrough (n=4)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>7.9±6.2</td>
<td>12.9±7.9</td>
<td>14.1±8.3</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2.3-26</td>
<td>4-23.1</td>
<td>3.7-24</td>
<td>0.07 (NS)</td>
</tr>
</tbody>
</table>

There was no statistically significant difference between IL-8 level in responders, non responders at 12 weeks and breakthrough at 24 weeks or between any pairs of these subgroups.

Pearson correlation co-efficient = 0.2 and p-value = 0.2 (Not significant) regarding the difference between IL-8 levels between groups according to baseline PCR levels.

ROC curve analysis for predictive characteristics of IL-8 in prediction of response to interferon therapy:

Area under the curve: 68%.
Standard error: 0.09.
95% confidence interval: 0.5-0.8.
p-value=0.04 (Statistically significant).

Value of IL-8≤6.7 has sensitivity of 74% (95% CI: 55.4-88.1), specificity of 64% (95% CI: 30.9-88.8), positive predictive value of 85%, negative predictive value of 47% and positive likelihood ratio of 2.04 and negative likelihood ratio of 0.4 in prediction of response (being responder) to interferon therapy.
Hepatitis C virus (HCV) is an infectious disease which affects about 170 million people worldwide [23]. Approximately 70-80% of infected individuals develop chronic infection and 20% of chronically infected individuals will go on to develop cirrhosis. Among these, 1-5% will develop end-stage liver diseases [24,25]. Currently, patients chronically infected with HCV are mainly treated with combination of pegylated interferon-α (IFN-α) plus ribavirin.

Several studies have been carried out to identify factors that facilitate identification of chronic hepatitis C patients who are likely to respond to antiviral treatment.

The HCV NS5A protein has been implicated in the resistance of HCV to antiviral therapy [26]. It was previously found that NS5A induces the CXC chemokine interleukin 8 (IL-8) to inhibit the antiviral actions of IFN in vitro [21]. To investigate the clinical significance of these results, in this study we investigated the impact of the levels of IL-8 in the serum and response to IFN therapy.

The aim of the present study was to determine the correlation between levels of interleukin-8 in serum of patients with chronic hepatitis C infection and response to pegylated interferon/ribavirin after 12 and 24 weeks of therapy respectively.

A total of 42 patients with chronic HCV infection were enrolled in the study. Patients were assessed for baseline laboratory characteristics and side effects to interferon therapy. Baseline interleukin-8 was measured before initiation of therapy. Virological response was assessed after 12 weeks and 24 weeks (as an endpoint).

The whole studied patients were in age group (20-58) years old, with mean age of 41.5 years old. About half of the studied patients were males (52.4%) and 47.6% were females. Most of the studied patients were from rural areas (61.9%).

The results of the present study come in accordance with what was recently reported by the Egypt Demographic and Health Survey. The Egypt Demographic and Health Survey (EDHS) estimated a prevalence of 14.9% for the sampled population of 11,126 aged 15-59 in 2008. Prevalence increased with age, with 55-59 year olds showing a rate of 39.4%. Overall prevalence was 17.4% in males and 12.2% in females [8]. Males had a modestly higher infection rate. Rural areas had a higher prevalence than urban [27].

At the end of 24 weeks of therapy, PCR showed that 73.8% of the studied patients have virologic response with negative PCR while 26.2% were positive (breakthrough), (16.7% at 12 weeks and 9.5% at 24 weeks).

There was no statistically significant difference between responders and non responders as regard to age, sex, residence and almost all of reported side effects to interferon/ribavirin therapy.

Baseline laboratory assessment showed that responders were found to have better controlled FBS, lower α-fetoprotein (2.8 versus 5.8 in non responders; p-value <0.05) and lower quantitative viral load (5.9 versus 36.8 in non responders; p-value <0.05).

Non responders were found to have higher baseline interleukin-8 levels than responders with mean IL-8 13.3±7.6pg/ml versus 7.9±6.2pg/ml respectively. Most of non responders were found to have IL-8 levels ≥14pg/ml (63.6%) while most of responders have IL-8 levels ranging from 2-6 pg/ml (74.2%).

The results of the current study are consistent with that of a previous study in 2001 by Polyak et al., [21] which was considered the first study to examine serum IL-8 levels and the biochemical response to IFN therapy. They have found that levels of IL-8 were significantly higher in patients who did not respond to IFN therapy than in patients who did respond to therapy.

In 2004, a study by Mihm and Colleagues [30] has investigated serum interleukin-8 in 59 healthy controls and 214 patients with chronic hepatitis C and different outcome to interferon-α-based therapy. In patients with chronic hepatitis C, higher interleukin-8 levels were observed compared with healthy controls (p<0.0001). They have also found that hepatitis C genotype 1-infected patients with early and overall virologic response to interferon-
α-based therapy showed lower interleukin-8 levels than non-responders (p=0.025 and p=0.035, respectively) that is consistent with the current findings.

Our findings can be supported by the conclusion that was stated by Jia and Colleagues in 2007 when they studied the mechanisms of IFN-α inhibition of HCV replication and the resistance of HCV to IFN-α therapy. Their study demonstrated that IFN-γ has synergistic antiviral effects with IFN-α; whereas IL-8 can attenuate the anti-HCV actions of IFN-α and is associated with HCV resistance to interferon-a therapy [31].

The findings of the current study are also consistent with the findings reported by most recent study by Akbar et al., (2011) [32] they aimed to prospectively utilize the baseline IL-8 levels in the HCV infected serum and predict its role in sustained virological response (SVR) to IFN-α plus ribavirin therapy, in chronic HCV patients in Pakistan. They have found that non responders have higher baseline pretreatment levels of IL-8 than responders. They have concluded that increased levels of IL-8 in HCV infection might be involved in pathogenesis, persistence and resistance to IFN-α plus ribavirin combination therapy.

In our study, baseline (pretreatment) IL-8 levels ≥14pg/ml were found to have relative risk of 3.1 (95% CI: 1.09-9.03) to be non responder while pretreatment IL-8 levels 2-6pg/ml show protective effect against being non responder with relative risk of 0.3 (0.1-0.9). Also, ROC curve analysis has shown that in patients with chronic HCV infection, pretreatment IL-8 value ≤ 6.7pg/ml have sensitivity of 74%, specificity of 64%, PPV of 85% and NPV of 47% in prediction of good virological response to interferon/ribavirin therapy.

We conclude that, low level IL-8 is a significant predictor for response to interferon/ribavirin therapy in patients with chronic HCV infection. Responders were found to have lower pre treatment serum levels of IL-8 than non responders. Also; responders have lower levels of base-line HCV RNA than non-responders. It is advisable to perform a larger scale study for more conclusive results.

References


