PROGNOSTIC VALUE OF SERUM SOLUBLE INTERLEUKIN-2 RECEPTOR IN EGYPTIAN CHRONIC HEPATITIS C PATIENTS TREATED WITH PEGYLATED INTERFERON AND RIBAVIRIN

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ABSTRACT

Background/Aim: Hepatitis C virus (HCV) is a major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma worldwide. A strong Th1 response seems to be associated with viral clearance. It is generally accepted that T cell activation is characterized by the synthesis and secretion of interleukin-2 and by the expression of Interleukin-2 receptors (IL-2R) on the cell surface of immune cells. Following lymphocyte activation, the alpha chain of the IL-2R mediates the interaction of IL-2 with its receptor. IL-2R is present in 2 forms: the high and low affinity receptors (sIL-2R). We aimed to determine the evolution of soluble IL-2 receptors (sIL-2-R), as an indicator of immune cell activation, in Egyptian chronic hepatitis C patients treated with pegylated interferon and ribavirin and its correlation with outcome of therapy.

Methods: 53 naïve (previously not treated) chronic HCV patients eligible for criteria of therapy according to the international guidelines were recruited. Pegylated interferon alpha-2a (IFNα-2a) was used subcutaneously once a week for 48 weeks. Ribavirin tablets in a dose of 13mg/kg were given daily in 2 divided doses every 3 month. Sera were collected at different time point before and during therapy and tested for level of soluble IL-2-R using ELISA techniques.

Results: Prior to therapy, mean serum soluble IL-2R level was significantly higher in patients with HCV as compared to controls (3709.05± 291.4 pg/ml versus 1770.6 pg/ml ±220.3, p<0.01). After end of therapy, patients were retrospectively classified into 2 groups, responders and non-responders. In responders, the level of sIL-2R raised significantly after 4 weeks of therapy as compared to pre-treatment level (4501 ±309 pg/ml versus 3550 ± 291 pg/ml p = 0.01). In Non-responders, however, the difference in serum sIL-2R before therapy and after 4 weeks of therapy was non-significant (4021 ±567 pg/ml versus 3934 ± 550 pg/ml p=0.9). The levels of serum sIL-2-R significantly correlated in a linear model with ALT levels before starting the therapy.

Conclusion: Monitoring of sIL-2R levels may therefore be of value as an adjunct to the measurement of serum ALT and HCV-RNA in predicting the response to interferon therapy in HCV patients.

Keyword: IL2, cytokines, chronic hepatitis, soluble IL2 receptors.

INTRODUCTION

Hepatitis C virus (HCV) is a major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma worldwide. The prevalence of HCV infection varies significantly; higher rates have been reported in African and Asian countries whereas industrialized nations in North America, Northern and Western Europe and Australia have lower prevalence rates (1). Egypt has a high prevalence rate of about 13.8% in the general population (2). HCV virus is not cytopathic, and the mechanism by which it causes liver injury is not well established (3). Immune response that is essentially conducted by cytokines may play an important role in the pathogenesis of HCV infection. A strong Th1 response characterized by the production of interleukin-2 (IL2), tumor necrosis factor – alpha (TNF-α) and interferon gamma (IFN-γ) seems to be associated with viral clearance. However, in the context of a persistent infection, they may be responsible for liver damage (4).

Combination therapy with pegylated alpha interferon (IFN-α) and ribavirin is currently standard treatment for patients with chronic hepatitis C. Viral clearance, however, is achieved in only 40 to 70% of patients. HCV genotype (5-7), the vigour of the T-cell proliferative response to hepatitis C virus (HCV) antigens in the acute phase of hepatitis (8), the mechanism by which pegylated IFN-α and ribavirin therapy induces resolution of chronic HCV infection is not fully understood. Nevertheless, since both drugs potentially have immunomodulatory activities in vivo, in addition to their antiviral properties, cellular immune responses against HCV may be involved in a successful treatment outcome (11-13). It is generally accepted that T cell activation is characterized by the synthesis and secretion of interleukin-2 and by the expression of IL-2 receptors (IL-2R) on the cell surface of immune cells. Following lymphocyte activation, the alpha chain of the high affinity IL-2R is released in soluble form (sIL-2R) in vivo and in vitro (16). The blood
sIL-2R levels depend on the number of producing cells and the number of molecules per cell, so that sIL-2R blood values may represent an index of the number and the functional state of producing cells. Since blood sIL-2R levels may correlate with disease progression and/or response to therapy, their measurement may be a useful index of activity and extent of disease. The aim of this study is to determine the evolution of sIL-2 receptors, as an indicator of activation of T cells in HCV patients treated with ribavirin and pegylated interferon and to correlate sIL-2 R serum levels with outcome of therapy.

SUBJECTS AND METHODS

Study Population:
Participants were recruited from clinics of the National Hepatology & Tropical Medicine Research Institute Cairo Egypt. They gave written informed consent under protocols approved by the Institutional Review Boards and compliant with guidelines of the Declaration of Helsinki regarding protection of human subjects. Only naïve patients eligible for criteria of therapy according to the international guidelines were recruited.

The diagnosis was based on elevated serum transaminase levels (ALT) and detection of anti-HCV antibodies by enzyme immunoassay and of HCV RNA by reverse transcription-PCR. Confirmation was obtained by liver histology. All patients were negative for hepatitis B virus surface antigen. In total, 53 participants were recruited.

Blood samples were collected immediately before therapy, at different time points during therapy (1, 3, and 6 months), and 6 months after the end of therapy.

Twenty age and sex matching participants with normal liver enzymes, negative for HCV Ab and HBsAg were taken as controls. Viral RNA Testing:
Plasma HCV viral RNA titers were measured using the Cobas Amplicor/ Cobas Tagman fully automated real time system with a lower detection limit of 15 HCV IU/ml (Roche Molecular Systems).

Soluble IL-2 receptor measurements:

sIL-2R concentrations were measured by sandwich ELISA technique using the commercially available sIL2R kit Diaclone France according to manufacturer instructions. The maximum detectable level of sIL2R by the kit was 2200 pg/ml. Samples were diluted 5 times with the supplied standard buffer diluents before working. The amount of sIL2-R in each sample was determined by extrapolating OD values to sIL2-R concentrations using a standard curve plotted using the used standards ranging from 68.75-2200 pg/ml. Final results were obtained by multiplication by the dilution factor.

Therapeutic method:
Pegylated interferon alpha-2a (IFNa-2a) was used subcutaneously once a week for 48 weeks. Ribavirin tablets in a dose of 13mg/kg were given daily in 2 divided doses. Liver function and complete blood picture were monitored weekly for the first month and then monthly in the course of administration of therapy. HCV-RNA was monitored every 3 month.

Statistical Analysis:
Statistical analyses were done by the SPSS software (Statistical Package for the Social Sciences version 9.0, SPSS Inc, Chicago, Ill, USA). Continuous variables were expressed as mean ± SE. The t test was used where applicable. P values less than 0.05 were considered of significance.

RESULTS

The study comprised 53 chronic HCV patients, 40 male and 13 female, their mean age was 39.6 ± 0.8 years. Mean viral load in HCV patients before therapy was 472,492±182,789 IU/ml. As regards ALT, mean level was 60 U/L, knowing that the upper limit of normal (ULN) used was less than or equal 40 U/L. Histopathological study of liver biopsy of all patients revealed a mean activity score of 5/18 and a mean fibrosis score of 2.1/6 using Ishak scoring system. Prior to therapy, mean serum soluble IL-2R level was significantly higher in patients with HCV as compared to controls (3709.05±291.4pg/ml versus 1770.6±220.3 pg/ml, p<0.01). Characteristics of the patients are shown in table(1)

Six months after the end of therapy, patients were retrospectively classified into 2 groups (according to the PCR results done at week 72): (a) Responders, those with a negative HCV viral load at the end of the therapy and which continued to be negative 6 months after cessation of treatment and (b) Non-responders, those with a positive HCV viral load at 48 weeks of therapy and for the sake of better comparison partial responders and relapers were also included in the non-responders group.

Accordingly, 42 patients were responders to therapy and had a sustained viral response (SVR) while 11 patients were non responders to therapy at different time points. No significant difference was found between responders and non-responders as regards...
mean age, ALT value, histopathological indices and viral load.

**Soluble IL2R values at different time points:**

In Responders, the level of sIL2R raised significantly after 4 weeks of therapy as compared to pre-treatment level (4501 ±309 versus 3709.05 ± 291.4 p= 0.02) and then started to decline progressively. Near normal levels were reached at 72 weeks (Table2).

In Non-responders, however, the difference in serum sIL2R before therapy and after 4 weeks of therapy was minimal (4021 ±567 versus 3709.05 ± 291.4 p=0.4), the level started to rise significantly only after 12 weeks of therapy (p= 0.004) and then declined progressively. In cases that were followed-up to 48 weeks, sIL2R levels never reverted to healthy control values (Table2).

The levels of serum sIL2-R significantly correlated in a linear model with ALT levels before starting the therapy r =0.6 (fig 1).

On the other hand, no correlation was found between viral load and serum sIL2-R prior to therapy (fig 2).

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**Table 1: Characteristics of patients before start of therapy.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of cases</td>
<td>53</td>
</tr>
<tr>
<td>Age in years (mean ±SE)</td>
<td>39.6 ± 0.8</td>
</tr>
<tr>
<td>Sex Male (No)</td>
<td>40</td>
</tr>
<tr>
<td>Sex Female (No)</td>
<td>13</td>
</tr>
<tr>
<td>ALT level before therapy (U/L) (mean ±SE)</td>
<td>60 ± 6.7</td>
</tr>
<tr>
<td>Histopathological Index (Activity Score)</td>
<td>5/18</td>
</tr>
<tr>
<td>Histopathological Index (Fibrosis Score)</td>
<td>2.1/6</td>
</tr>
<tr>
<td>HCV RNA value before therapy (IU/ml) (mean ±SE)</td>
<td>472,492 ± 182,798</td>
</tr>
<tr>
<td>sIL-2R (pg/ml) (mean ±SE)</td>
<td>3709.05 ± 291.4*</td>
</tr>
</tbody>
</table>

* p<0.05 compared to controls

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**Table 2: sIL2R values (pg/ml) in HCV patients at different time points during therapy**

<table>
<thead>
<tr>
<th>Serum sIL2R (pg/ml)</th>
<th>Controls (n=20)</th>
<th>All Patients (n=53)</th>
<th>Responders (n=42)</th>
<th>Non-Responders (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>1770.6±</td>
<td>3709.05 ± 291.4*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Week 4</td>
<td>-</td>
<td>4501 ±309**</td>
<td>4021 ±567</td>
<td></td>
</tr>
<tr>
<td>Week 12</td>
<td>-</td>
<td>3907±256</td>
<td>5779 ±584***</td>
<td>(n=11)</td>
</tr>
<tr>
<td>Week 24</td>
<td>-</td>
<td>2056±107</td>
<td>4345 ±195 (n=7)</td>
<td></td>
</tr>
<tr>
<td>Week 48</td>
<td>-</td>
<td>1895±264</td>
<td>3899 ±274 (n=4)</td>
<td></td>
</tr>
<tr>
<td>Week 72</td>
<td>-</td>
<td>1230±304 (n=35)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

* p<0.05 compared to controls

**p < 0.05 compared with patients at day 0

*** p< 0.05 compared with patients at day 0

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**Figure 1: Correlation between serum sIL2-R and serum ALT levels r=0.6**
HCV frequently sets up persistence, although the mechanisms that allow the virus to coexist with its host and develop chronic infection are not fully understood. The tendency of HCV-infected patients to produce a strong Th-1 response, characterized by the production of interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-a) and interferon gamma (IFN$\gamma$) seems to be associated with HCV clearance and it is believed that a deficit in Th-1 response could favor HCV chronicity\(^{(18)}\). It is currently known that the level of serum sIL2-R facilitate monitoring of T-lymphocyte activity and its serial measurements may aid in assessing disease progression\(^{(17)}\).

In the current study we have shown that prior to interferon therapy, the levels of sIL2-R were significantly higher in chronic HCV patients than in healthy controls. Similar results were obtained by Hayashi et al\(^{(19)}\) & Wang et al\(^{(20)}\). Pre-treatment levels of sIL2-R correlated with ALT levels but did not correlate with HCV RNA levels. Similar results were reported by Naveau et al.\(^{(21)}\).

Evidence exists showing that the clinical efficacy of IFN-alpha therapy in chronic HCV infection depends on the induction of HCV specific Th-1 cell response\(^{(22)}\). In the current study, after 4 weeks of therapy, the levels of sIL2-R were significantly higher than pre-treatment levels in responders and then started to decrease gradually. There was a normalization of sIL2-R levels as compared to healthy controls at week 72. Similar results were obtained by Kawakami et al,\(^{(23)}\) Hayashi et al\(^{(19)}\) & Quiroga et al\(^{(20)}\). In non-responders, levels of sIL2-R at week 4 did not significantly increase compared to pre-treatment levels, but at week 12 of therapy the level started to increase significantly and then started to decline gradually, but never reached control values. From these findings it can be argued that the rapid increase in sIL2-R level reflecting a rapid Th-1 response after the start of interferon and ribavirin is favorable for the response to therapy, while a slow rise in sIL2-R level lead to inability of the host to eradicate the virus. The reduction of serum sIL2-R level in responders may have been due to a reduction in inflammation after eradication of the virus and the failure to reduce its level in non-responders may mean that the reduction of inflammation was incomplete and T-lymphocytes were still activated leading to continuous liver damage. Therefore monitoring of serum sIL2-R in HCV patients treated with interferon and ribavirin at various time points during therapy and especially after 4 weeks from the start of treatment may facilitates the prediction of the effectiveness of therapy.

In conclusion, monitoring of sIL2-R levels may therefore be of value as an adjunct to the measurement of serum ALT and HCV-RNA in predicting the response to interferon therapy in HCV patients. Further study is needed to evaluate the use of immunmodulators and immunostimulants in order to activate the immune system in patients with low sIL2-R level early in the course of therapy giving them a better chance of response to therapy.

REFERENCES


الملخص العربي

القيمته التشخيصية لمستويات مستقبلات انترلوكن 2 الذاتية في دم المرضى المصريين المصابين بالالتهاب الكبدية الوبائي سي® والمعالجين بالانترفيرون طويل المفعول والريبافيرين

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الالتهاب الكبدية الوبائي سي هو أحد الأسباب الرئيسية لمرض التهاب الكبد المزمن، تليف الكبد وسرطان الخلايا الكبدي. يستلزم الارتفاع الشديد في مستوى استجابة الخلايا Th-1 مع اكتمال فيروس من الدم، من المعرفة عليه أن تنشط كرات الدم البيضاء من النوع 4، يجعله تكوين وارتفاع مستقبلات انترلوكن 2. وكذلك طورت مستقبلات انترلوكن 2 على جدار الخلايا المناعية، ثم ذوبتها بالماء.

يهدف هذا البحث إلى دراسة مستويات مستقبلات انترلوكن 2 الذاتية في دم مرضى التهاب الكبد الحاد في قبول بعده العلاج وطول فترة العلاج ودراسة الاختلاف في المستوى على توجه العلاج. ضمت هذه الدراسة عدد 53 مريض مصاب بالالتهاب الوبائي سي لم يسبق علاجهم. ثم علاج هذه الحالات بالانترفيرون طويل المفعول حقنه في الأسبوع لمدة 48 أسبوع وفاز بداء وريبيفايرين يوميا. ثم أخذ عينات دم لملاحظ مستويات مستقبلات انترلوكن 2 الذاتية في فترات زمنية مختلفة قبل وأثناء العلاج باستخدام تقنية الالعاب.

أوضح التحليل أن مستوي مستقبلات انترلوكن 2 الذاتية كانت أعلى بكثير لدى المرضى مقارنة بالعينات المأخوذة من المجموعة الضابطة. في نهاية فتر العلاج قسم المرضى إلى مجموعتين: استجيبا و غير استجيبا. في المجموعة المستجيبا رتفع مستوي مستقبلات انترلوكن 2 الذاتية بوضوح بعد 4 أسابيع من العلاج مقارنة بمستويات ما قبل العلاج. أما في المجموعة غير المستجيبا للعلاج كان التبيان ضئيل في المرضى بعد 4 أسابيع من العلاج ولم يبلغ المستوى بوضوح إلا بعد 12 أسبوع من العلاج. مما سبق يوضح أن قياس مستوي مستقبلات انترلوكن 2 الذاتية في مرضى التهاب الكبد الوبائي سي بعد أربع أسابيع من بدء العلاج قد يدل إذا كان المريض سوف يستجيب للعلاج أم لا وذلك بالإضافة إلى قياس إنزيمات الكبد واختبار البليسي.