OBJECTIVE ARTICLE

Serum KL-6 Level is Elevated in Chronic Hepatitis C Patients with Combination Therapy of Pegylated Interferon and Ribavirin

Yasuji Arase1, Kenji Ikeda1, Yoshiyuki Suzuki1, Masahiro Kobayashi1, Fumitaka Suzuki1, Norio Akuta1, Hitomi Sezaki1, Tetsuya Hosaka1, Hiromi Yatsuji1, Yusuke Kawamura1, Mariko Kobayashi2 and Hiromitsu Kumada1

Abstract

Objectives The aim of this study was to evaluate whether or not combination therapy of pegylated interferon (IFN) and ribavirin for chronic hepatitis (CH) C patients enhances the serum level of KL-6, a sensitive marker for interstitial pneumonia.

Methods CH C patients proven histologically and treated with combination therapy of pegylated IFN-alpha-2b and ribavirin, IFN monotherapy or untreated for 48 weeks were retrospectively selected in chronological order in groups of 25. Serum levels of KL-6 were measured by enzyme-linked immunosorbent assay by use of serum stored at -80°C before and at 12, 24, 36, 48 weeks after the initiation of treatment or follow up.

Results The average serum KL-6 levels in patients treated with combination therapy of pegylated IFN and ribavirin increased by 21% at 12 weeks after the start, 23% at 24 weeks, and 28% at 48 weeks. In patients treated with combination therapy of pegylated IFN and ribavirin, the serum KL-6 level significantly increased during treatment. Patients achieved an elevated serum KL-6 level of more than 450 U/ml with statistical significance when: 1) combination therapy was given (P=0.011), 2) serum KL-6 level pretreatment was high more than 300 U/ml (P=0.014).

Conclusion The present study suggests that onset of interstitial pneumonia should be carefully checked in the combination therapy of pegylated-IFN and ribavirin.

Key words: interstitial pneumonia hepatitis C virus, KL-6, interferon, ribavirin

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Introduction

Chronic hepatitis (CH) C infection can be associated with progressive liver disease that may evolve insidiously into liver cirrhosis and carries an increased risk of hepatocellular carcinoma (HCC) (1). The majority of deaths due to HCC are ascribed to hepatitis viruses, of which 70-80% corresponding to approximately 30,000 per year is attributed to the persistent infection with HCV in Japan (2, 3). Thus, it is important to eradicate HCV-RNA or decrease levels of alanine aminotransferase (ALT) for preventing HCC with interferon (IFN) therapy (4, 5). Recently, it has been reported that novel long-acting formulations of IFN known as induce higher eradication rate of HCV (6-8).

However, IFN therapy has been associated with various IFN-related adverse events. The onset of interstitial pneumonia (IP) has been reported when HCV positive patients were given IFN (9-11). Moreover, Karim et al (12) reported that the new regimen of combination therapy of pegylated IFN and ribavirin causes IP. However, it is not clear how much each treatment for chronic hepatitis C induces IP. IP remains a disease of unknown etiology with a poor prognosis after acute exacerbation. It can progress rapidly after such exacer-

1 Department of Hepatology, Toranomon Hospital, Tokyo and 2 Hepatic Research Unit, Toranomon Hospital, Tokyo
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Correspondence to Dr. Yasuji Arase, es9y-ars@asahi-net.or.jp

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bation and often proves fatal, despite treatment with oral corticosteroids and intravenous high-dose corticosteroid therapy. Thus, in order to arrest exacerbation of IP, it is necessary to achieve early diagnosis and early treatment.

Serum KL-6 level is a sensitive marker of disease activity in fibrosing lung diseases (13). Hirawasa et al reported that KL-6 is one of the chemotactic factors for most fibroblasts and that the increased KL-6 in the epithelial lining fluid in small airways may cause the intra-alveolar fibrosis in fibrosing lung disease (14). KL-6 is a high-molecular-weight glycoprotein and is classified as MUC1 mucin of lung tumor and differentiation antigens. The molecule consists of multiple heterogeneous submolecules. KL-6 can be detected by the murine monoclonal antibody (mAb), KL-6 antibody (IgG1), which recognizes a sialylated sugar chain on the molecule. Our previous studies indicate that changes in the serum KL-6 level can provide useful information to assess early diagnosis of idiopathic pulmonary fibrosis (IPF) (15).

Thus, we retrospectively assessed changes in serum KL-6 level in HCV patients treated with combination therapy of IFN plus ribavirin and IFN monotherapy.

Materials and Methods

Patients

The number of patients who were diagnosed as having chronic HCV infection by laparoscopy and liver biopsy at the study hospital between 1989 and 2005 was 4,600. We divided these patients into the following four groups. One group of 480 patients was given combination therapy pegylated IFN-alpha-2b and ribavirin for one year (group 1). Patients in group 1 were given pegylated IFN-alpha-2b (Schering-Plough Corp., Kenilworth, NJ) subcutaneously at a dosage of 100 μg (for those with body weight of ≥60 kg) or 80 μg (for those with body weight of <60 kg) weekly for one year and ribavirin (Rebetol; Schering-Plough Corp) at an oral dose of 600 mg (for patients with body weight <60 kg) or 800 mg (for those with body weight of ≥60 kg) daily for 48 weeks. A second group of 222 were treated with natural IFN-alpha (Sumitomo Pharmaceutical Co., Osaka, Japan) for > one year (group 2). Patients in group 2 received IFN subcutaneously at a dosage of 6 million units (MU) daily for eight weeks, followed 3 times weekly for > 44 weeks. A third group of 650 patients was followed for more than one year without treatment. The remaining patients were given treatments other than those described above.

We selected patients in chronological order in groups of 25 from the first to the third groups described above. These patients were consecutively selected when they had the following criteria: 1) positivity for HCV-RNA by amplicor monitor assay (16) or reverse transcription nested polymerase chain reaction (RT-nested PCR) (17); 2) HCV-genotype 1b; 3) no treatment with corticosteroid, immunosuppressive agents, or antiviral agents within 12 months; 4) negativity for hepatitis B surface antigens, antinuclear antibodies, or antimitochondrial antibodies in the serum, as determined by radioimmunoassay and spot hybridization; 5) leukocytes > 3,000/mm³, platelets >80,000/mm³ and bilirubin <2.0 mg/ml.

We excluded from the present study all of the following patients: 1) those with liver cirrhosis or HCC, 2) with a history of malignant tumor, 3) whose serum level of KL-6 showed high level of >400 U/L. This study was approved by the institutional ethics review board of our hospital. The physician in charge explained the purpose and method of this clinical trial to each patient, who later gave his/her informed consent for participation.

Blood testing

Follow-up began on the first day of initiation of treatment in patients given IFN with ribavirin or without. On the other hand, follow-up began on the day of liver biopsy in untreated patients. The serum samples of patients divided into three groups were collected before and at 3, 6, 9, and 12 months after the start of follow-up. The samples were stored at -80°C until the time of assay for KL-6. The serum concentration of KL-6 antigen was measured by a sandwich-type enzyme-linked immunosorbent assay (ELISA) using immobilized KL-6 antibody (Eitest®, KL-6, Eisai, Tokyo, Japan) as described previously (14). A serum KL-6 level of > 450 U/ml during follow-up was defined as an elevated KL-6 group. Unelevated KL-6 group was defined as no elevated KL-6 level during follow-up.

Moreover, using these blood samples, HCV-RNA levels before follow-up were analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) (18). HCV genotype was examined by a PCR assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously (19).

Liver histology

Liver biopsy specimens were obtained percutaneously or by peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin-eosin, Masson’s trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas. Histopathological interpretations of these 3-to 4-μm thick sections were made independently by experienced liver pathologists (YA and HK) who had no clinical information or knowledge of chronological order of the biopsies in each pair. Baseline liver histopathology of chronic hepatitis prior to IFN therapy was classified according to the extent of fibrosis, into three stages: mild (periporal expansion); moderate (portoportal septa); and severe (portocentral linkage or bridging) fibrosis (20).

Statistical analysis

Clinical differences among three groups before follow-up were assessed by the Kruskal Wallis test. Changes in serum
Table 1. Clinical Characteristics of the Patients Enrolled

<table>
<thead>
<tr>
<th></th>
<th>Combination therapy group</th>
<th>IFN monotherapy group</th>
<th>Untreated group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>23</td>
<td>25</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>53 (30-62)</td>
<td>51 (18-67)</td>
<td>50 (32-65)</td>
<td>.32</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>18/5</td>
<td>13/2</td>
<td>17/8</td>
<td>.13</td>
</tr>
<tr>
<td>Liver fibrosis (mild/moderate/severe)</td>
<td>12/9/2</td>
<td>15/8/2</td>
<td>18/5/2</td>
<td>.093</td>
</tr>
<tr>
<td>HCV-RNA (KIU/mL)*</td>
<td>890 (100-2100)</td>
<td>980 (22-5000)</td>
<td>1100 (45-2400)</td>
<td>.39</td>
</tr>
<tr>
<td>KL-6 (U/mL)</td>
<td>204 (125-428)</td>
<td>210 (90-397)</td>
<td>229 (120-398)</td>
<td>.075</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>59 (32-191)</td>
<td>68 (38-348)</td>
<td>36 (26-86)</td>
<td>&lt;.0001</td>
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<tr>
<td>ALT (IU/L)</td>
<td>97 (46-299)</td>
<td>210 (46-397)</td>
<td>58 (19-137)</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Data are number of patients or median (range)  
ALT = alanine aminotransferase; AST = aspartic aminotransferase; HCV = hepatitis C virus; IFN = interferon

KL-6 level measured every 12 weeks were calculated as percent changes from the pretreatment baseline in each patient. Differences between pretreatment baseline and percent change at each time point in each group were compared by a Wilcoxon rank sum test. Differences among three groups were compared by a Wilcoxon rank sum test, too. Clinical differences between elevated KL-6 group and unelevated KL-6 group were evaluated by Wilcoxon rank sum test or Fisher’s exact test. A P value <0.05 was considered to be statistically significant. The Statistical Program for Social Sciences software package (SPSS Inc., Chicago, 11) was used to perform statistical analysis.

Results

Pretreatment clinical characteristics

Two of 25 patients treated with combination of pegylated IFN and ribavirin dropped out from this clinical study owing to IFN-related adverse effect of depression and general fatigue. Thus, the remaining 23 patients treated with combination of pegylated IFN and ribavirin were evaluated. A total of 50 patients belonging to the other two groups were followed for 48 weeks without dropout. Table 1 shows the pretreatment baseline characteristics in patients of all three groups enrolled in this study. There were no differences among the three groups in factors other than the serum level of transaminase. The serum levels of AST and ALT in patients untreated were significantly lower than those in patients treated of remaining three groups.

Changes in serum KL-6 level during the clinical course

Changes in serum KL-6 levels during follow-up were shown in Fig 1. In patients treated with combination therapy of pegylated IFN and ribavirin, the comparison with the baseline value (0 weeks) disclosed an increase in the KL-6 level at 12, 24, 36, and 48 weeks with statistical significance. The average serum KL-6 levels increased to 21% at 12 weeks after the start, 23% at 24 weeks, and 28% at 48 weeks. In patients treated with IFN monotherapy, the KL-6 levels did not show an increase at 12, 24, 36, or 48 weeks with statistical significance.

Factors contributing to the elevated serum KL-6 level

Six of 73 patients showed an elevated serum KL-6 level of more than 450 U/ml. Table 2 shows clinical differences between elevated KL-6 group and unelevated KL-6 group. Patients achieved an elevated serum KL-6 level with statistical significance when: 1) combination therapy was given (P=0.011), 2) serum KL-6 level pretreatment was high more than 300 U/ml (P=0.014).

Discussion

Serum KL-6 levels are generally increased in patients with fibrosing lung infection (13, 14). However, serum levels of KL-6 are sometimes elevated in patients with certain malignancies such as adenocarcinoma of the lung, breast, pancreas and HCC (21, 22). In the present study, none of the patients had malignancies for two years after the initiation of follow-up. Therefore, we think that changes in the serum KL-6 level in the present study were not related to malignancies.

The present study indicates the following. First, serum levels of KL-6 in HCV-positive patients untreated remained stable during follow-up. Second, the serum levels of KL-6 in patients treated with combination of pegylated IFN and ribavirin were significantly increased compared to those of pretreatment. About 20% of patients treated with combination therapy of pegylated IFN and ribavirin showed an ele-
Figure 1. Change over time in serum KL-6 levels compared with pretreatment values in chronic hepatitis C patients receiving each treatment. Changes are expressed as mean percentages ± standard deviation of pretreatment baseline values for each group. *Serum KL-6 levels of group treated with combination therapy of pegylated IFN and ribavirin were significantly greater compared to that of pretreatment (P<0.05). ^P = comparison of KL-6 level between patients treated with pegylated IFN and ribavirin and untreated. ^P = comparison of KL-6 level between patients treated with IFN monotherapy and untreated.

Table 2. Difference of Backgrounds between Elevated KL-6 Group and Un-elevated Group

<table>
<thead>
<tr>
<th></th>
<th>Elevated KL-6 group</th>
<th>Uncalated KL-6 group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>6</td>
<td>67</td>
<td>.739</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45(39-60)</td>
<td>52(18-67)</td>
<td>.088</td>
</tr>
<tr>
<td>IFN therapy</td>
<td>6</td>
<td>42</td>
<td>.011</td>
</tr>
<tr>
<td>Combination therapy</td>
<td>5</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Sex(male/female)</td>
<td>4/2</td>
<td>45/21</td>
<td>.100</td>
</tr>
<tr>
<td>Liver fibrosis</td>
<td>3/2/1</td>
<td>44/29/1</td>
<td>.388</td>
</tr>
<tr>
<td>KL-6(U/ml) before</td>
<td>317(187-371)</td>
<td>202(90-398)</td>
<td>.035</td>
</tr>
<tr>
<td>follow-up</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KL-6(U/ml) &lt;300≥300</td>
<td>2/4</td>
<td>56/11</td>
<td>.014</td>
</tr>
<tr>
<td>AST(U/L)</td>
<td>50(43-64)</td>
<td>56(26-339)</td>
<td>.801</td>
</tr>
<tr>
<td>ALT(U/L)</td>
<td>76(49-115)</td>
<td>79(19-383)</td>
<td>.779</td>
</tr>
<tr>
<td>HCV-RNA(KIU/ml)</td>
<td>560(100-2900)</td>
<td>680(45-&gt;5000)</td>
<td>.208</td>
</tr>
</tbody>
</table>

Data are number of patients or median (range)

ALT = alanine aminotransferase; AST = aspartic aminotransferase; HCV = hepatitis C virus; IFN = interferon

Elevated serum KL-6 level of > 450 U/ml. We think that the combination therapy of pegylated IFN with ribavirin has a possibility to cause IP. Third, patients had an elevated serum KL-6 level with statistical significance when combination therapy was given and the serum KL-6 level at the time of pretreatment was higher than 300 U/ml. Regrettably, in the present study, six patients with > 450 U/ml were not examined for IP by computed tomography in details because they did not have complaints of severe cough or difficulty of breathing. Thus, the onset of IP was not confirmed in the present study.

IP is one of the well-known IFN-related side effects (9-
Although IFN-induced IP rarely occurs, it is sometimes fatal. The serum level of KL-6 is elevated in the majority of patients with various interstitial lung diseases, including IP. The present study indicates that the combination therapy of pegylated IFN and ribavirin significantly increased the serum KL-6 level. Therefore, when physicians give combination therapy of pegylated IFN and ribavirin to the patients with hepatitis C, they should be aware of IP onset. At that time, analysis of the serum KL-6 level is beneficial in detecting early diagnosis of IP.

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References


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