Clinical Significance of KL-6, a Marker of Interstitial Pneumonia, in Cases of HCV-associated Chronic Liver Disease

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Abstract

Objective Various antiviral therapies, including interferon therapy, are being conducted to treat chronic hepatitis C and suppress the onset of hepatocellular carcinoma. However, interstitial pneumonia is beginning to be recognized as one of the adverse reactions of this therapy, and is one of the complications associated with chronic hepatitis. Therefore, we measured the level of KL-6, an interstitial pneumonia marker, in patients with HCV-associated chronic disease, and then determined the possibility of utilizing serum KL-6 as a predictive factor for interstitial pneumonia and the clinical significance of KL-6 in HCV-associated chronic disease.

Subjects and Methods The subjects were 308 patients who were diagnosed with chronic liver disease through biochemical blood tests and abdominal diagnostic imaging. All patients tested positive for either the HCV antibody or HCV-RNA, and those who were suspected of having autoimmune hepatitis were excluded. One hundred eighty-five patients had chronic hepatitis (average age: 56±14 years), while 123 patients had liver cirrhosis (average age: 64±9 years). The purpose of the present study was explained to every subject, and informed consent was obtained.

Results The mean KL-6 level for chronic hepatitis patients without interstitial pneumonia was 283.5±131.4 U/ml, while that for cirrhotic patients without interstitial pneumonia was significantly higher, at 377.6±212.1 U/ml (p<0.0001). In addition, with a cut-off value of 500 U/ml, the ratio of high KL-6 for the chronic hepatitis patients was 5.41% (10/185), while that for the cirrhotic patients was significantly higher, at 20.33% (25/123) (p<0.0001). Furthermore, the mean KL-6 level for patients with a serum hyaluronic acid level of less than 100 ng/ml was 258.4±124.6 U/ml, while that for patients with a serum hyaluronic acid level of 100 ng/ml or above was significantly higher, at 381.0±197.3 U/ml (p<0.0001).

Conclusion Although KL-6 is a marker of interstitial pneumonia, the results of the present study suggest that, in HCV-associated chronic disease, this marker reflects hepatic fibrosis better than pulmonary fibrosis.

Key words: chronic liver disease, HCV, interstitial pneumonia, hepatic fibrosis

Introduction

Because only approximately 10% of patients with interstitial pneumonia (IP) test positive for the HCV antibody (1), HCV may not necessarily cause interstitial pneumonia (IP), but IP has been considered as an extrahepatic complication of HCV-associated chronic disease. In addition, while HCV induces fibrosis in the liver, because the underlying condition of IP is pulmonary fibrosis, HCV may correlate with IP. Furthermore, there have been reports of IP caused by drugs used in the treatment of liver disease. Of the various drugs used in the treatment of liver disease, Sho-sai-koto has been shown to cause IP (2), and because interferon has been actively used in the treatment of chronic hepatitis C, IP caused by interferon has attracted attention in recent years (3).

Interferon is essential in improving the QOL of patients with HCV-associated chronic disease. As a result, it is extremely important to be able to predict the onset of IP in order to provide proper care for patients with HCV-associated chronic disease. In this study, we measured the level of KL-6, a serum marker for IP, to determine whether
KL-6 could serve as a predictive factor for IP and to investigate the clinical significance of KL-6 in HCV-associated chronic disease.

**Subjects and Methods**

**Subjects**

The subjects were 308 patients and inpatients with HCV-associated chronic disease who were treated at the Department of Gastroenterology and Hepatology, University Hospital at Koshigaya Dokkyo University School of Medicine between 1999 and 2001. Chronic liver disease was diagnosed through biochemical blood tests and abdominal diagnostic imaging, and hepatitis C was confirmed by a positive reaction to either the HCV antibody (Second generation) or HCV-RNA (RT-PCR). One hundred eighty-five patients, 95 men and 90 women with an average age of 56±14 years, had chronic hepatitis C, while 123 patients, 68 men and 55 women with an average age of 64±9 years, had liver cirrhosis. Of the 123 patients, 95 had compensatory liver cirrhosis, while 28 had decompensated liver cirrhosis.

Hepatocellular carcinoma was confirmed in 3 of the 185 patients with chronic hepatitis C (1.6%) and in 24 of the 123 patients with liver cirrhosis (19.5%). Of the 308 patients, 104 patients (33.7%) were taking Chinese medications, while 14 patients (4.5%) were receiving interferon therapy. Of the 222 patients with reported smoking habits, 4 patients (1.8%) smoked more than 20 cigarettes per day.

**Methods**

Among these patients, biochemical tests results, the levels of serum hyaluronic acid (sandwich binding protein assay) and type III procollagen N-terminal peptide (RIA) were measured. The level of serum KL-6 was measured using A TEST KL-6 (Eisai, Tokyo). When the level of KL-6 was above a cut-off value of 500 U/ml, chest CT was performed on consenting patients, and CT scans were examined for IP by radiologists.

**Statistical analysis**

Values were expressed as mean±S.D. Comparison between two groups was analyzed using unpaired t-test. Variable frequency was compared using Fisher’s exact test. Correlations between each variable were tested using Pearson’s correlation coefficient. To evaluate the association between high serum KL-6 (>500 U/ml) and potential risk factors, odds ratio and corresponding 95% CI were computed by means of unconditional logistic regression models. A value of p<0.05 was considered statistical significant.

**Results**

**Serum KL-6 levels in HCV-associated chronic disease**

Of the 308 patients, 4 patients had IP. Figure 1 shows the serum KL-6 level for 304 patients without IP. The mean KL-6 level for the 184 patients with chronic hepatitis C was 283.5±131.4 U/ml, while that for the 120 patients with liver cirrhosis was significantly higher, at 377.6±212.1 U/ml (p<0.0001). In addition, the severity of liver cirrhosis was assessed according to Child’s classification system into two groups: Grade A and Grade B/C. The KL-6 level for 86 patients (Grade A) was 334.0±177.8 U/ml, while that for 54 patients (Grade B/C) was significantly higher, at 425.9±220.2 U/ml (p<0.0081). The ratio of patients with a KL-6 level above the cut-off value was 5.41% (10/185) for the chronic hepatitis-C patients, while that for the cirrhotic patients was significantly higher, at 20.33% (25/123) (p<0.0001).

**Correlation to hepatic fibrosis markers**

In order to clarify the correlation between KL-6 and advances in HCV-associated chronic diseases, particularly hepatic fibrosis, the level of two hepatic fibrosis markers, hyaluronic acid (HA) and Type III procollagen N-terminal peptide (P-III-P), was measured (Fig. 2). The mean serum KL-6 level for the 113 patients with a serum HA level of less than 100 ng/ml was 283.5±131.4 U/ml, while that for the 120 patients with liver cirrhosis was significantly higher, at 377.6±212.1 U/ml (p<0.0001). In addition, the severity of liver cirrhosis was assessed according to Child’s classification system into two groups: Grade A and Grade B/C. The KL-6 level for 86 patients (Grade A) was 334.0±177.8 U/ml, while that for 54 patients (Grade B/C) was significantly higher, at 425.9±220.2 U/ml (p<0.0081). The ratio of patients with a KL-6 level above the cut-off value was 5.41% (10/185) for the chronic hepatitis-C patients, while that for the cirrhotic patients was significantly higher, at 20.33% (25/123) (p<0.0001).
Figure 3. Correlation between serum level of KL-6 and hyaluronic acid.

than 100 ng/ml was 258.4±124.6 U/ml, while that for the 144 patients with a serum HA level of 100 ng/ml and above was significantly higher, at 381.0±197.3 U/ml (p<0.0001). In addition, the mean serum KL-6 level for the 150 patients with a serum P-III-P level of less than 1.5 U/ml was 332.8±179.1 U/ml, while that for the 10 patients with a serum P-III-P level of more than 1.5 U/ml was 439.9±208.2 U/ml, but there was no significant difference between the two groups (p=0.0718).

Serum HA and KL-6 levels

With patients who might have had IP (serum KL-6: >500 U/ml) excluded, the close relationship between serum HA and KL-6 was investigated (Fig. 3). A significant positive correlation was found between serum HA and KL-6 (p=0.0002).

Chronic liver disease markers related to KL-6

KL-6 is a glycoprotein antigen that was once used as a lung cancer marker, but is currently being used as an IP one. As a result, a logistic analysis was performed among patients with chronic liver disease to potential risk factors related to serum KL-6 (Tables 1 and 2). Among the 304 patients with HCV-associated chronic disease without IP, a univariate analysis was performed, and the results showed that the OR for demonstrating high serum KL-6 (>500 U/ml) was 5.5 with high HA (>100 ng/ml, 95%CI: 2.0–14.7), 3.4 with liver cirrhosis (95%CI: 1.6–7.4), 2.7 with liver cirrhosis (95%CI: 1.2–5.8), 2.7 with low platelet count (<10x10^7/μl, 95%CI: 1.2–5.4), but 0.2 with chronic hepatitis (95%CI: 0.1–0.5). A multivariate analysis including these parameters showed a significant correlation with high HA (>100 ng/ml) only (OR: 3.4). No significant correlations were seen with the other parameters, such as Chinese herbal medication or hepatocellular carcinoma.

Serum KL-6 and hyaluronic acid levels in patients with HCV-associated chronic disease with IP

Of the 308 patients, 4 patients had HCV-associated chronic disease complicated by IP (Table 3). One patient was

### Table 1. Univariate Analysis for All Factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95%CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver cirrhosis</td>
<td>3.386</td>
<td>1.550–7.399</td>
<td>0.0022</td>
</tr>
<tr>
<td>Chronic hepatitis</td>
<td>0.248</td>
<td>0.113–0.543</td>
<td>0.0005</td>
</tr>
<tr>
<td>Chinese herbal medicine</td>
<td>0.506</td>
<td>0.200–1.281</td>
<td>0.1506</td>
</tr>
<tr>
<td>HCC</td>
<td>2.487</td>
<td>0.923–6.699</td>
<td>0.0715</td>
</tr>
<tr>
<td>Child A</td>
<td>0.526</td>
<td>0.210–1.313</td>
<td>0.1684</td>
</tr>
<tr>
<td>AST/ALT (&gt;1)</td>
<td>2.664</td>
<td>1.219–5.821</td>
<td>0.0140</td>
</tr>
<tr>
<td>P-III-P (≥1.5 U/ml)</td>
<td>1.714</td>
<td>0.435–6.754</td>
<td>0.4410</td>
</tr>
<tr>
<td>Hyaluronic acid (≥100 ng/ml)</td>
<td>5.481</td>
<td>2.033–14.777</td>
<td>0.0008</td>
</tr>
<tr>
<td>Platelet (10x10^7/μl)</td>
<td>2.599</td>
<td>1.243–5.435</td>
<td>0.0112</td>
</tr>
</tbody>
</table>

HCC: heptocellular carcinoma, P-III-P: type III procollagen N-terminal peptide.

### Table 2. Multivariate Analysis for Significant Factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95%CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver cirrhosis</td>
<td>1.753</td>
<td>0.669–4.594</td>
<td>0.2533</td>
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<tr>
<td>HCC</td>
<td>1.215</td>
<td>0.397–3.712</td>
<td>0.7331</td>
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<tr>
<td>AST/ALT (&gt;1)</td>
<td>1.704</td>
<td>0.723–4.015</td>
<td>0.2231</td>
</tr>
<tr>
<td>Hyaluronic acid (≥100 ng/ml)</td>
<td>3.393</td>
<td>1.122–10.263</td>
<td>0.0305</td>
</tr>
<tr>
<td>Platelet (10x10^7/μl)</td>
<td>1.231</td>
<td>0.500–3.034</td>
<td>0.6511</td>
</tr>
</tbody>
</table>

HCC: heptocellular carcinoma.

### Table 3. Patients with HCV-associated Chronic Liver Diseases Complicated by Interstitial Pneumonia

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Age</th>
<th>Sex</th>
<th>KL-6 U/ml</th>
<th>HA ng/ml</th>
<th>Interferon</th>
<th>Chinese herbal medicine</th>
<th>Smoking</th>
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</thead>
<tbody>
<tr>
<td>LC</td>
<td>61</td>
<td>M</td>
<td>907</td>
<td>680</td>
<td>none</td>
<td>Without</td>
<td>none</td>
</tr>
<tr>
<td>LC</td>
<td>75</td>
<td>F</td>
<td>1,260</td>
<td>135</td>
<td>none</td>
<td>Without</td>
<td>none</td>
</tr>
<tr>
<td>CH</td>
<td>66</td>
<td>M</td>
<td>282</td>
<td>96</td>
<td>none</td>
<td>Without</td>
<td>none</td>
</tr>
<tr>
<td>LC</td>
<td>55</td>
<td>M</td>
<td>293</td>
<td>92</td>
<td>none</td>
<td>With</td>
<td>none</td>
</tr>
</tbody>
</table>

LC: Liver cirrhosis, CH: Chronic hepatitis, HA: Hyaluronic acid.
taking Sho-sai-koto, and while it was not clear whether this drug caused interstitial pneumonia, the other three patients were not taking any medication that could have induced IP. Of the 4 patients with IP, the level of serum KL-6 was above the cut-off value in 2 patients (50%). The level of serum KL-6 was markedly high in 1 patient, and was below the cut-off value in 2 patients.

**Changes in KL-6 in a patient with a high KL-6 level before interferon therapy**

Of the 14 patients receiving interferon therapy, the level of serum KL-6 was high before interferon therapy in 1 patient. Figure 4 shows the chronological changes in the levels of serum KL-6 and HA in this patient. After the interferon therapy, the levels of serum KL-6 and HA decreased during progress, and although chest CT was performed twice, no clear signs of IP were seen.

**Discussion**

Although hepatocellular carcinoma and esophageal varices are widely recognized as complications associated with HCV-associated chronic disease, IP has not been well recognized as an extrahepatic complication associated with hepatitis-C virus infection, due to its low incidence. Ever since Sho-sai-koto, a drug that was once widely used in the treatment of HCV-associated chronic disease, was reported to cause IP, there has been greater interest in drug-induced IP. In addition, in recent years, interferon has been actively administered to patients with chronic hepatitis C in order to prevent cancer, and interferon-induced IP is attracting greater attention as a result. While the incidence of interferon-induced IP is low (0.4%, 3/677) (5), it is one of the clinically significant adverse reactions associated with this therapy. Therefore, it is clinically useful to be able to predict the onset of drug-induced IP when administering Sho-sai-koto or interferon to patients with chronic hepatitis C. As far as drug-induced IP is concerned, one clinical study on Sho-sai-koto reported increased lymphocytes and neutrophils and a decreased CD4/8 ratio in the bronchoalveolar lavage fluid (BALF) (4). In addition, one study found that the incidence of IP among patients with hepatitis-C virus infection (8/66, 12.2%) was higher than that among healthy volunteers (3.66%) (1), thus suggesting a close relationship between hepatitis-C virus infection and IP. Another study analyzed the BALF of patients with chronic hepatitis C, and reported that while the total cell count and the number of lymphocytes for the hepatitis patients were higher than those for healthy individuals, there was no significant difference in CD4/8 ratio (6). These BALF findings were similar to those associated with drug-induced IP. This data suggests that patients with hepatitis-C virus infection are prone to IP, and that allergic reactions to a drug can induce IP in some patients.

KL-6, a glycoprotein antigen discovered by Kohno et al (7), and that is currently being used as an IP marker. In addition, KL-6 is a monoclonal antibody (IgGl) that recognizes the carbohydrate section of a glycoprotein antigen, which is expressed abundantly in type-II alveolar epithelial cells (7), is a potent chemotactic factor for fibroblasts (8), and facilitates pulmonary fibrosis. Furthermore, the level of KL-6 in the BALF of interstitial pneumonia patients was significantly higher than that of healthy control subjects (9) and, with interstitial pneumonia, when alveolar epithelial, basal, and endothelial cells are damaged, the air-blood barrier is compromised and, when permeability accelerates, KL-6/MUC1 is absorbed into the blood, thus elevating its serum concentration. However, in the present study, the level of serum KL-6 for the liver cirrhosis patients was significantly higher than that for the chronic hepatitis patients and, when liver cirrhosis was classified according to Child’s system, the level of serum KL-6 for the Child B/C patients was significantly higher than that for the Child A patients, thus suggesting a correlation between KL-6 and liver disease. In addition, serum HA is a marker that reflects the severity of hepatic fibrosis (10), and there was a significantly positive correlation between HA and KL-6. Furthermore, P-III-P is a marker that reflects fibrosis progression, but is not a marker for the grading of fibrosis in the hepatic tissue. As the level of serum KL-6 was high among patients with a high level of serum P-III-P and there was a positive correlation between HA and KL-6, it appears that high KL-6 is correlated with increased fibrosis progression and hepatic fibrosis. In the present study on HCV-associated chronic disease, the ratio of patients with a serum KL-6 level above the cut-off value was 11.4% (35/304), which was clearly higher than a healthy volunteer reported by Kobayashi et al (1.83%, 5/273) (11). Among the 35 patients with a serum KL-6 level above the cut-off value, only 4 patients showed clear signs of IP and, as the incidence of IP among patients with a serum KL-6 level above the cut-off value has been reported at 81%, the incidence of IP...
among the patients with HCV-associated chronic disease was clearly lower. These findings suggest that KL-6 in patients with HCV-associated chronic disease reflects hepatic fibrosis better than pulmonary fibrosis.

Among the patients receiving interferon therapy, the level of serum KL-6 was high in one patient before therapy and, in this patient, the levels of serum HA and KL-6 decreased following the therapy. In addition, this patient did not have the complication of IP during the study. Therefore, in order to clarify the use of KL-6 as a predictive factor for interstitial pneumonia, it will be necessary to follow patients taking interferon and patients in stable condition for an extended period.

References