Clinical Manifestations of HBsAg and Anti-HCV Negative Chronic Liver Disease in Nagasaki Prefecture, Japan

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The discovery of hepatitis C virus (HCV) has enabled the diagnosis of type C chronic liver disease, which had in the past been diagnosed as part of non-A, non-B chronic liver disease. Although most cases with chronic liver disease are caused by hepatitis B virus (HBV) or HCV infection, there are still cases of non-B, non-C chronic liver disease. Forty patients with chronic liver disease, who were seronegative for hepatitis B surface antigen and antibody to HCV, were followed for a mean period of 72 months. The clinical manifestations in these patients were compared with those reported for type B and type C chronic liver disease. Of the 40 patients, 22 were diagnosed with chronic hepatitis, 14 with liver cirrhosis and 4 with hepatocellular carcinoma (HCC). Twenty-seven (67.5%) patients showed mild alanine aminotransferase activity profiles, and the natural clinical course of most patients showed a slow progression compared with that reported for type B and type C patients. The yearly incidence of HCC was 9.7% in patients with liver cirrhosis and 3.9% in chronic hepatitis. These rates were similar to those in type B or type C patients. This suggests that our population sample contained a number of patients with type B, type C or other etiologic agent(s), because 66.7% of the patients who developed HCC had some evidence of exposure to HBV or HCV. Our results suggest that more detailed and accurate tests for detecting HBV and HCV should be considered before making the diagnosis of non-B, non-C chronic liver disease, and that there is the need to reveal unknown etiologic agent(s).

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**Key words:** non-B, non-C chronic liver disease, liver cirrhosis, hepatocellular carcinoma, long-term prognosis

Introduction

Following the discovery of hepatitis C virus (HCV) as the major cause of non-A, non-B hepatitis (1), most cases with chronic liver disease, including chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC), were found to be caused by hepatitis B virus (HBV) or HCV infection, including cases reported in Nagasaki Prefecture (2), where the HBV carrier rate is higher than in other parts of Japan (3).

Although reliable immunoassays and molecular probes for detecting HBV and HCV are currently available (4, 5), it is still difficult to completely rule out the presence of HBV and HCV infections. Moreover, 10–20% of non-A, non-B hepatitis cases are caused by non-C, non-E etiologic agent(s) (6) which has (have) remained to be clarified. Kodali et al (7) also reported that the underlying mechanism of approximately 5% of patients with chronic hepatitis/cirrhosis is unknown despite the use of HCV-RNA testing. Therefore, yet unidentified non-B, non-C agent(s) might be implicated in chronic liver disease (8). But the natural course of non-B, non-C chronic liver disease has been seldom described.

In the present study, we examined the clinical manifestations of patients diagnosed with hepatitis B surface antigen (HBsAg) and antibody to the hepatitis C virus (anti-HCV) negative chronic liver disease in Nagasaki Prefecture, Japan. We also compared their features with those reported for type B
Patients and Methods

A total of 40 patients with HBsAg and anti-HCV negative chronic hepatitis, liver cirrhosis or HCC, including 25 females and 15 males (range: 12–88 years, mean, 60 years), were registered between April 1974 and October 1993 in the Nagasaki Hepatitis Study Group according to the following criteria: 1) The patient exhibited elevated levels of serum aminotransferases, and was followed for at least 6 months. 2) The patient was seronegative for HBsAg, and for anti-HCV confirmed by enzyme immunoassay or radioimmunoassay (second generation). 3) Patients with high titres of antibody to the hepatitis B core antigen (anti-HBc) were excluded from the study. 4) The diagnosis of chronic hepatitis and liver cirrhosis was based on histologic and/or peritoneoscopic findings, while the diagnosis of HCC was based on histological and/or angiographic examination. 5) Patients with drug-induced liver injury, those with a history of chronic alcohol consumption (>70 g/day for 5 years or more), patients with fatty liver diagnosed by characteristic findings on ultrasonography and/or computed tomography, and patients with autoimmune hepatitis or primary biliary cirrhosis were also excluded from the present study.

The activity pattern of alanine aminotransferase (ALT) during the follow-up period was classified into 6 types, shown schematically in Fig. 1. Type 1 was characterized by the presence of normal or slightly elevated levels of ALT (less than twice the upper normal limit) throughout the follow-up period. Type 2 showed fluctuating high levels of ALT activity in the initial phase of the disease with subsequent return to a normal level. Type 3 represented abnormally high levels of ALT (less than three times the upper normal limit) throughout the follow-up period. Type 4 was similar to type 3 but the level of ALT was persistently less than five times the upper normal limit. Type 5 represented abnormally high levels of ALT (more than five times the upper normal limit) throughout the follow-up period. Finally, type 6 was characterized by fluctuating levels of ALT activity with the low levels approaching normal values while the high levels exceeded three times the upper normal limit.

The observation period commenced with enrolment and all patients were followed regularly at participating hospitals; the study period was terminated at the end of April 1994. Of the 40 patients, 4 were lost to follow-up and 4 died during the study.

Values were expressed as mean±SD. Statistical analysis was performed using the chi-square test or Fisher's exact probability test (two-tailed). A p value of less than 0.05 was considered significant.

Figure 1.  Schematic diagram of ALT activity patterns.
Results

The follow-up period extended over a mean period of 72.2±60.7 months, ranging from 6 to 216 months. The diagnosis was established histopathologically in 32 patients, including chronic persistent hepatitis in 11 patients, chronic active hepatitis with peripoortal necrosis in 8, chronic active hepatitis with bridging necrosis in 3, liver cirrhosis in 9 and HCC in one patient. The diagnosis of liver cirrhosis was established in another 5 patients using peritoneoscopy alone, while 3 patients were diagnosed with HCC based on the characteristic appearance on ultrasonography, computed tomography and angiography.

Examination of the serum at enrolment showed values above the normal limits for bilirubin in 7 (18%) patients, aspartate aminotransferase in 22 (55%) and ALT in 19 (48%) patients. Antibody to HBsAg (anti-HBs) was positive in 11 of 28 patients, and anti-HBc was positive with a low titre in 7 of 17 patients. None of the 10 patients tested for HCV RNA in blood using the polymerase chain reaction (PCR) test, and none of the 26 patients tested for antinuclear antibody by the indirect immunofluorescence, gave a positive result. Five of the 40 patients (13%) had received blood transfusions in the past and 7 (18%) gave a family history of liver diseases. Of these 40 patients, 2 patients had a history of angina pectoris, 6 diabetes mellitus, 7 hypertension, 3 gall stones, 1 duodenal ulcer and 1 patient had chronic thyroiditis.

**ALT activity patterns**

Of these 40 patients, 9 (22.5%) patients showed an ALT profile of type 1, 6 (15%) showed type 2, 12 (30%) showed type 3, 6 (15%) showed type 4, 7 (17.5%) showed type 6, and no patient showed type 5 (Fig. 1).

**Long-term outcome**

The long-term outcome for 34 patients is shown in Fig. 2. The outcome in 30 patients was not examined histologically but clinically mainly due to a hemorrhagic tendency in 21 cirrhotic patients with or without HCC. Of these 34 patients, the disease process did not change in 18 (52.9%) patients (4 patients with liver cirrhosis and 14 chronic hepatitis) during a mean follow-up period of 57.5 months (non-progression group). Although there was no significant correlation between the ALT profile and the long-term outcome, patients showing an ALT profile of type 1 were more common in the progression group (Table 1). Interestingly, 4 of 6 patients with an ALT profile of type 1 in the progression group developed HCC. A larger population of patients with liver cirrhosis [5 (55.6%) of 9 patients] developed HCC compared with patients with chronic hepatitis [4 (18.2%) of 22 patients] during a mean follow-up period of 63.4 and 82.0 months, respectively.

**Incidence of hepatocellular carcinoma**

During the follow-up period, HCC was recognized in 9 of 34 (26.5%) patients, based on histological and/or angiographic findings. Of these 9 cases, 5 were initially diagnosed with liver cirrhosis, 3 with chronic active hepatitis, and 1 with chronic persistent hepatitis. Therefore, the incidence of HCC was 55.6% (5 of 9) in liver cirrhosis and 18.2% (4 of 22) in chronic hepatitis. In our population sample, the yearly incidence of

<table>
<thead>
<tr>
<th>Table 1. Relationship between ALT Activity Pattern and Clinical Progression</th>
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<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-------------------------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Progression group (n=16)</td>
</tr>
<tr>
<td>Non-progression group (n=18)</td>
</tr>
</tbody>
</table>

*p=0.1128 (two-tailed Fisher’s exact probability test). **Four of 6 patients developed hepatocellular carcinoma (HCC). ALT: alanine aminotransferase.
Non-B, Non-C, Chronic Liver Disease

HCC was 5.4%, with 9.7% in patients afflicted with liver cirrhosis and 3.9% in patients with chronic hepatitis. Although there was no correlation between the ALT profiles and the incidence of HCC, the latter developed even in 4 patients with type 1 ALT profile characterized by a persistently normal level of ALT (Table 2). Of these 4 patients, 2 were seropositive for both anti-HBs and anti-HBc, 1 was seropositive for anti-HBs and seronegative for anti-HBc. Another patient was not tested for either anti-HBs or anti-HBc. Of the remaining 5 patients who developed HCC, 1 had received blood transfusion, 1 was seropositive for anti-HBs, and 1 was seropositive for anti-HBc. Therefore, only 3 patients (33.3%) gave no evidence of exposure to etiologic factors relevant to viral hepatitis. The clinical background of these 9 patients who developed HCC is summarized in Table 3.

The positivity rate of anti-HBs and anti-HBc in all patients tested and in patients who developed HCC was 39.3% (11 of 28) and 41.2% (7 of 17), 57.1% (4 of 7) and 42.9% (3 of 7) respectively. There was no correlation, in general, between seropositivity of anti-HBs or anti-HBc and the likelihood of developing HCC.

### Table 2. Relationship between ALT Activity Pattern and Incidence of HCC

<table>
<thead>
<tr>
<th>ALT type</th>
<th>No. of patients</th>
<th>Incidence of HCC*</th>
<th>Follow-up period months (mean)</th>
<th>Yearly incidence of HCC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>4</td>
<td>6–192 (56.4)</td>
<td>12.2</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>0</td>
<td>6– 84 (31.7)</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>3</td>
<td>6–216 (80.2)</td>
<td>4.1</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>1</td>
<td>7– 17 (11.7)</td>
<td>34.2</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>1</td>
<td>6–132 (61.4)</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*p=0.1880 (two-tailed Fisher’s exact probability test). ALT: alanine aminotransferase, HCC: hepatocellular carcinoma.

### Table 3. Clinical Background of 9 Patients who Developed HCC

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>Age</th>
<th>Sex</th>
<th>Initial Diag.</th>
<th>Follow-up period (Mo)</th>
<th>ALT type</th>
<th>Anti-HBs</th>
<th>Anti-HBc</th>
<th>BT</th>
<th>FH</th>
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<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>M</td>
<td>LC</td>
<td>12</td>
<td>A</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>73</td>
<td>F</td>
<td>LC</td>
<td>6</td>
<td>A</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>81</td>
<td>M</td>
<td>CH</td>
<td>23</td>
<td>A</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>69</td>
<td>M</td>
<td>CH</td>
<td>192</td>
<td>A</td>
<td>NT</td>
<td>NT</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>67</td>
<td>M</td>
<td>LC</td>
<td>216</td>
<td>C</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>77</td>
<td>F</td>
<td>LC</td>
<td>48</td>
<td>C</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>M</td>
<td>CH</td>
<td>17</td>
<td>D</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>77</td>
<td>F</td>
<td>LC</td>
<td>38</td>
<td>C</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>61</td>
<td>M</td>
<td>CH</td>
<td>96</td>
<td>F</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
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</table>


### Discussion

The criteria selected for enrolment in the present study did not exclude the presence of HBV or HCV infection since the detection of serum HBV-DNA or HCV-RNA by PCR technique was not performed. Uchida et al (9) reported that the PCR successfully amplified serum HBV-DNAs in 85% of non-B, non-C, non-D chronic hepatitis. De Mitri et al (10) also analyzed liver tissues and serum samples from 19 patients negative for HBsAg by PCR. Their results demonstrated that 13 and 7 patients were HCV-RNA and HBV-DNA positive in liver tissue, respectively. Moreover, several reports have recently provided evidence for other agents of human non-A, non-B, non-C, non-D, non-E hepatitis (6, 11, 12). Therefore, it is likely that our population sample contained a number of patients with type B, type C or other etiologic agent(s). However, since it is still difficult to rule out these cases entirely even after the use of the PCR technique, it is important to examine the clinical manifestations of cases diagnosed with non-B, non-C chronic liver disease based on conventional serological markers of HBV and HCV.

Of the 40 patients, 27 (67.5%) exhibited ALT profiles of type 1, 2 or 3, representing a mild ALT activity. These ALT profiles were milder than those of type C chronic liver disease (13, 14),
which reveal waxing and waning patterns. On the other hand, 7 (17.5%) patients showed type 6, characterized by fluctuation of ALT activity. Of these, 3 had a family history of liver diseases and were suspected of HBV or HCV infection. The natural course of our 40 patients generally showed a slow progression compared with that of reported cases of type B and type C chronic liver disease (15). While some patients showed a rapid progression, there was no correlation, in general, between the ALT profile and the long-term outcome.

We previously reported the risk of HCC in patients with liver cirrhosis in Nagasaki Prefecture (16), the cumulative risk of HCC in non-B, non-C cirrhotic patients was significantly lower than that in type B or type C groups. In the present study, however, the yearly incidence of HCC was 9.7% in HBsAg and anti-HCV negative cirrhotic patients, which was higher than that reported previously (16). This high incidence may be influenced by the presence of HBV or HCV infection in our patients, since 6 (66.7%) of the 9 patients who developed HCC were suspected to have been exposed in the past to HBV or HCV (Table 3). Interestingly, 5 of the 7 tested patients were seropositive for anti-HBs and/or anti-HBc, suggesting that these patients were infected in the past by HBV. HCC may develop after inactivation of HBV replication in HBV carrier patients. HBV carrier state has some direct influence on hepatocarcinogenesis, because the integration of HBV has been detected in almost all patients with HCC, mainly attributed to transcriptional transactivating activity of the X gene (17). Although we did not test for HBV-DNA in our patients, it is likely that the high incidence of development of HCC was, in part, attributed to HBV.

In conclusion, the ALT activity pattern and long-term progression of HBsAg and anti-HCV negative chronic liver disease were milder than those of type B or type C chronic liver diseases. However, the clinical course in a number of patients was similar to that of type B or type C chronic liver disease, including a high incidence of HCC (18–20). Our findings indicate that more detailed and accurate tests for detecting HBV and HCV, e.g., combined one-step PCR method to detect both HCV-RNA and HBV-DNA in a single serum specimen (21), should be considered before making the diagnosis of non-B, non-C chronic liver disease.

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References


