Hepatitis C Virus Infection on Iki Island, Japan, an Area Endemic for Human T-lymphotropic Virus Type-I. A Preliminary Study in Patients at Clinics or Hospitals

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Patients undergoing routine blood tests at clinics or hospitals on Iki Island, Japan were tested for antibody to hepatitis C virus (anti-HCV), hepatitis B surface antigen (HBsAg), and antibody to human T-lymphotropic virus type-I (anti-HTLV-I). Anti-HCV accounted for 17.5%, HBsAg for 2.9%, and anti-HTLV-I for 30.3%. Anti-HCV was present in 60.2% of the patients with liver disease and in 13.5% of those without liver disease. A history of blood transfusion was a statistically significant risk factor for both HCV and HTLV-I infections, as was a history of acupuncture in case of HCV infection. Of the people surveyed, 7.5% had both anti-HCV and anti-HTLV-I, and anti-HTLV-I-positive persons had a significantly higher prevalence of anti-HCV than did anti-HTLV-I-negative persons (24.8% vs 14.3%, p<0.05). These data suggest that Iki Island is highly endemic for both hepatitis C virus (HCV) and for human T-lymphotropic virus type-I (HTLV-I). Blood transfusion may have been the common route in the spread for both HCV and HTLV-I. Acupuncture also may have facilitated the spread of HCV infection, in this area. J Epidemiol, 1994; 4 : 17-23.

HCV, HBV, HTLV-I, epidemiology, acupuncture

Hepatitis C virus (HCV), hepatitis B virus (HBV), and human T-lymphotropic virus type-I (HTLV-I) are the most significant blood-borne viruses causing malignant diseases. Most cases of hepatocellular carcinoma (HCC) are caused by HCV or HBV and HTLV-I is associated with adult T-cell leukemia (ATL). HTLV-I has a geographic clustering and the southwestern part of Japan is one of the most highly endemic areas in the world. HBV is also endemic in this area, and localized endemic areas of HCV have also been recently identified. Iki Island is an isolated island in Nagasaki prefecture, located in the southwestern part of Japan. Nagasaki prefectural statistics show that Iki Island has extremely high mortality rates of HCC and leukemia/lymphoma; 38.8/100,000/year and 40.9/100,000/year, respectively. In attempts to eradicate these malignant diseases, measures must be taken against the causative viral infections; both by implementing strategies against the routes of infection and by treating infected people. However, the magnitude of HBV, HCV, and HTLV-I infections on this island had not heretofore been assessed.

We investigated the prevalences of these viral infections, seroepidemiologically, as the initial step in trying to eliminate HCC and ATL on Iki Island. This is a preliminary report.

MATERIALS AND METHODS

Study Population

Patients at clinics/hospitals on Iki Island were surveyed for the prevalence of HCV, HBV, and HTLV-I infections during 1992, through the cooperation of the Iki Medical Association, Iki Regional Health Conference, and the Department of General Medicine, Kyushu University Hospital. Iki Island is located about 25 km north to the main island of Kyushu (Figure 1), with a population of approximately 37,000. The chief industries are agriculture, fishing, and the tourism. Eight of 17 clinics and two
of three hospitals on Iki Island participated in this study (Table 1). Special clinics for the treatment of liver or hematological diseases were not included.

A total of 1,106 serum samples were collected, mainly from outpatients, undergoing routine blood tests between January and February, 1992; these were 455 males, 651 females; age 5 to 59 years, with a mean of 59 years. A questionnaire for each patient concerning age, gender, residence, birthplace and diagnosis was filled out by the staff of each clinic and sent with the collected sera to the Department of General Medicine, Kyushu University Hospital, where all the samples were tested. A history of medical treatment including blood transfusion, surgery and acupuncture was acquired by direct mail.

All samples were stored at -20°C and tested for antibody to HCV (anti-HCV), HBV surface antigen (HBsAg), and antibody to HTLV-I (anti-HTLV-I).

**Assay Methods**

Anti-HCV was tested using the second generation anti-HCV test by passive hemagglutination assay (PHA) (HCVPHA, Dainabot, Tokyo, Japan). The procedure involves the use of three recombinant antigens; pHCV-34, pHCV-31, and pHCV-31. The inhibition test was done on specimen reactive by PHA, using recombinant HCV antigen, with those reactive by this test considered to be positive for anti-HCV.

HBsAg was tested using reversed passive hemagglutination assay (RPHA) (REVERSECELL, Megroken, Osaka, Japan).

Anti-HTLV-I was tested using the gelatin particle agglutination (PA) test (SERODIA-ATLA, Fujirebio, Tokyo, Japan), with specimen reactive by the PA test confirmed by western blots, using antigens prepared from MT-2 cells. For the western blots, the proteins, p19, p24, p28, and gp68 were checked, with sera showing reactivity to p19 plus at least two of the three remaining proteins considered positive. Specimen reactive by western blot analysis were considered to be positive for anti-HTLV-I.

**Statistical Analysis**

Differences in proportions were compared using the chi-square test or the Mantel-Haenszel test. A p value of less than 0.05 was considered to have statistical significance.

**RESULTS**

Iki Island is separated into four townships (Figure 1). The survey rates in the four areas ranged from 1.8 to 5.6%, mean 3.1%, of the estimated population. Distribution of the patients surveyed by township and clinic is shown in Table 1. Information on the history of blood transfusion, surgery, and acupuncture was obtained from 61.8% of the people surveyed. The mean age and the number of people with a history of blood transfusion or surgery were high in town K, while the rate of acupuncture was high in town G. Age distribution of people was lower in town I than in the other three towns, because town I has the pediatric clinic (clinic No. 6).

Table 2 shows the geographic distribution of the prevalences of anti-HCV, HBsAg, and anti-HTLV-I. The overall prevalence of anti-HCV was 17.5%, HBsAg 2.9%, and anti-HTLV-I 30.3%. The prevalence of anti-HCV was significantly higher in area G (26.2%) than was found in the other three areas (7.5-10.8%) (p<0.001). The prevalence of anti-HTLV-I was lower in area I (24.3%) than it was in the other three areas (30.6-33.2%), with significant differences between areas K and G, and area I (p<0.001). The prevalences of HBsAg in the four areas were from 2.7 to 3.1%, but with no statistically significant differences.

Table 3 shows the prevalence of anti-HCV, HBsAg, and anti-HTLV-I according to diagnosis. The diseases were classified according to WHO ICD-9-CM. Of the 93 people in whom liver disease was diagnosed, anti-HCV was detected in 60.2% and HBsAg in 10.8%. The prevalence of anti-HCV and HBsAg among persons with dis-
orders other than liver disease was 13.5% and 2.2%, respectively. Anti-HTLV-I was detected in 8(53.3%) of the 15 people diagnosed as having neoplasms. All four patients diagnosed as having leukemia were positive for anti-HTLV-I.

Age- and gender- specific prevalence of anti-HCV, HBsAg, and anti-HTLV-I is shown in table 4. The prevalence of anti-HCV tended to increase with age, except for the 50-59 age group. The prevalence of anti-HCV was nearly 20% in the 40-79 age groups, and reached almost 30% in the >80 age group, however, anti-HCV was not detected in age groups under 20 years. The prevalence of anti-HTLV-I also showed a tendency to increase with age; from <5% to >40%, except for the <10 group in which the number studied was small. In contrast, no correlation was found between age and the prevalence of HBsAg. The overall prevalence of anti-HTLV-I was significantly higher in females (33.9%) than in males (25.1%) (p<0.05 by
Table 3. Prevalence of anti-HCV, HBsAg, and anti-HTLV-I among clinic patients according to diagnosis: Iki, Japan, 1992.

| Diagnosis                                      | No. tested | anti-HCV (%) | HBsAg (%) | anti-HTLV-I (%) |
|------------------------------------------------|------------|--------------|-----------|-----------------|
| Infectious diseases                            | 7          | 14.3         | 14.3      | 28.6            |
| Neoplasms                                      | 15         | 13.3         | 0         | 53.3            |
| Endocrine, nutritional, and metabolic disease   | 82         | 13.3         | 2.4       | 36.6            |
| and immunity disorders                         | 24         | 8.3          | 8.3       | 12.5            |
| Mental disorders                               | 9          | 33.3         | 0         | 12.1            |
| Diseases of the nervous system and sense organs| 16         | 6.3          | 6.3       | 50.0            |
| Diseases of the circulatory system             | 411        | 17.8         | 1.7       | 34.5            |
| Disease of the respiratory system              | 131        | 9.2          | 1.5       | 20.6            |
| Diseases of the digestive system               | 154        | 42.2         | 7.1       | 35.1            |
| Liver diseases                                 | 93         | 60.2         | 10.8      | 44.1            |
| Others                                         | 61         | 14.8         | 1.6       | 21.3            |
| Diseases of genitourinary system               | 13         | 7.7          | 7.7       | 30.8            |
| Complications of pregnancy, childbirth, and    | 2          | 0            | 0         | 0               |
| puerperium                                     |            |              |           |                 |
| Diseases of the skin and subcutaneous and      | 9          | 0            | 0         | 33.3            |
| connective tissue                              |            |              |           |                 |
| Diseases of the musculoskeletal system         | 42         | 14.3         | 0         | 38.1            |
| Injury and poisoning                           | 7          | 0            | 0         | 42.9            |
| Others                                         | 15         | 0            | 6.7       | 13.3            |
| Health examination                             | 169        | 9.5          | 2.4       | 17.2            |
| Total                                          | 1,106      | 17.5         | 2.9       | 30.3            |

The Mantel-Haenszel test, while the overall prevalences of anti-HCV and HBsAg were higher in males, but with no statistical significance.

Table 5 shows the prevalence of HCV and HBsAg by anti-HTLV-I status. Eighty-three (7.5%) of the 1,106 people tested were positive for both anti-HTLV-I and anti-HCV and nine (0.8%) for both anti-HTLV-I and HBsAg. Anti-HTLV-I-positive people showed a significantly higher prevalence of anti-HCV than did anti-HTLV-I-negative people (24.8% vs 14.3%; p<0.05 by the Mantel-Haenszel test). However, no correlation was found between HBsAg and HTLV-I status.

A correlation between the history of possible percutaneous exposure through medical treatments and the prevalence of anti-HCV and anti-HTLV-I is shown in Table 6. The prevalence of anti-HCV was significantly higher among those with a history of blood transfusion (29.1%, p<0.001) or acupuncture treatment (23.8%, p<0.05). Prevalence of anti-HCV was also higher among people with a history of surgery than among those without, but with no statistically significant difference. Prevalence of anti-HTLV-I was significantly higher among people with a history of blood transfusion (43.1%, p<0.01), but no correlation was found between the prevalence of anti-HTLV-I and the history of operation and acupuncture.

Table 7 shows findings in transfused persons, based on the antibody status of HCV and HTLV-I. Of the four groups classified, the rate was highest in the anti-HCV(+)/anti-HTLV-I(+) group, with a significant difference between anti-HCV(+)/anti-HTLV-I(+) and anti-HCV(-)/anti-HTLV-I(-) (p<0.001).

DISCUSSION

We found high prevalences of both anti-HCV (17.5%) and anti-HTLV-I (30.3%) on Iki Island, Japan. The incidence of anti-HCV and anti-HTLV-I among blood donors in the Tokyo area of Japan was reported to be 1.2% (4), and 0.7% (5), respectively. Since our subjects were patients at clinics or hospitals with a high mean age, the prevalences may not always reflect the actual prevalences among the general population in the area studied. However, patients other than those with liver diseases or neoplasms did have a high prevalence of anti-HCV and anti-HTLV-I, with the comparison of prevalences by age being much higher among the people surveyed on Iki Island than were noted in Tokyo. Iki is a highly endemic area for both HTLV-I and HCV.

Of the people surveyed 7.5 percent were infected with both HCV and HTLV-I, and anti-HTLV-I-positive persons had a significantly higher prevalence of anti-HCV than did anti-HTLV-I-negative persons. The results suggest at least two possibilities: The first is that HTLV-I infected people are subject to HCV infection. Correlations...
between HTLV-I and Strongyloides stercoralis have been reported, suggesting impairment of immunity among HTLV-I carriers permits the proliferation and detection of the parasite, or the reverse\(^15\). Similarly, HTLV-I infection may be a cofactor which promotes HCV infection. However there is no evidence to support this, and the results of reports from other HTLV-I-endemic areas in Japan\(^16\) and our results from Yonaguni Island of Okinawa.
sterilization, in case a doctor makes house calls, the result of treatment. Since disposable instruments were formerly available, medical treatment may have been a risk factor for HCV infection. Far from it, the same needles were sometimes used for one person after another without sterilization, in case a doctor makes house calls, the result being percutaneous exposure. The lowest prevalence of anti-HCV in town K of the four towns, despite the highest rate of blood transfusion, may be related to the fact that doctors in town K had not made house calls so frequently as doctors in the other towns had. The high prevalence of anti-HCV in older age groups, especially in those over age 40, may be due to unsophisticated method of medical treatment in the past, while the existence of localized HCV-endemic areas in Japan may be related to former regional medical circumstances.

In contrast to the older age groups, there were no anti-HCV-positive individuals under age 20 years. This is consistent with previous reports (14,23) that suggested a low incidence of vertical transmission of HCV. Further study concerning familial transmission of HCV will be documented soon (Nakashima et al, submitted).

In conclusion, Iki Island is highly endemic for HCV and HTLV-I, but not for HBV. The distribution of HCV is related to age, history of blood transfusion or acupuncture, and to the antibody status of HTLV-I. In addition to blood transfusion, methods used in handling instruments in the past may be related to the high prevalence of anti-HCV in the area studied.

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**Table 7. Rate of transfused persons among clinic patients according to anti-HTLV-I and anti-HCV status: Iki, Japan, 1992.**

| Anti-HCV | Anti-HTLV-I | No. | Blood transfusions |
|---------|-------------|-----|--------------------|
| (++)    | (++)        | 47  | 16 (34.0)          |
| (+)     | (-)         | 63  | 18 (28.6)          |
| (-)     | (+)         | 160 | 34 (21.3)          |
| (-)     | (-)         | 414 | 49 (11.8)          |

Total 684 117 (20.2)

* p<0.01 by the chi-square test.

**Table 8. Rate of transfused persons among clinic patients according to anti-HTLV-I and anti-HCV status: Iki, Japan, 1992.**

| Anti-HCV | Anti-HTLV-I | No. | Blood transfusions |
|---------|-------------|-----|--------------------|
| (++)    | (++)        | 47  | 16 (34.0)          |
| (+)     | (-)         | 63  | 18 (28.6)          |
| (-)     | (+)         | 160 | 34 (21.3)          |
| (-)     | (-)         | 414 | 49 (11.8)          |

Total 684 117 (20.2)
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