ORIGINAL CONTRIBUTION

An Epidemiologic Survey of Hepatitis C Virus in Japan

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From 1988-91 we surveyed the prevalence of hepatitis C virus (HCV) infection among the general population of Japanese individuals living in Fukuoka and Okinawa, Japan. The combined antibody to c100-3 protein (anti-c100) and the antibody to GOR epitope (anti-GOR) were used. Persons with HCV infection had anti-c100 and/or anti-GOR in their sera. The overall rate of HCV infection was 3.7% of 2,835 persons in Fukuoka, a rate significantly higher than the 0.7% of 1,789 in Okinawa (p<0.001). There was no association between gender and prevalence. In Fukuoka, the prevalence of HCV infection increased with age, from 0.6% in the 0-9 age group to 11.5% in those over 70 years of age. In the over 50 age group, episodes of blood transfusion (18.6%) and surgery (56.7%) in the general population of Fukuoka were more frequent than those (7.0%, 38.6%, respectively) of Okinawa (p<0.001). Moreover, the blood for transfusion in Fukuoka was supplied by paid donors at commercial blood banks from 1953 to 1969, but in Okinawa was supplied by relatives and friends. These results suggest that the difference in prevalence of HCV infection between Fukuoka and Okinawa may be due to episodes of medical treatments and that blood for transfusion from paid donors played an important role in HCV infection. J Epidemiol, 1993; 3 : 41-45.

antibody to c100-3 protein, antibody to GOR epitope, blood transfusion, general population, hepatitis C virus infection

The genome of a non-A, non-B hepatitis virus termed hepatitis C virus (HCV) was molecularly cloned and identified as a positive-stranded RNA molecule1). An enzyme-linked immunosorbent assay (ELISA) was developed to detect antibody to the protein (c100-3), as expressed in cloning experiments2). Although the antibody to c100-3 (anti-c100) has been widely used for epidemiologic studies, anti-c100 does not cover all HCV infections and hepatitis C occurred in some patients who were transfused with anti-c100-negative blood3). Such being the case, we used both anti-c100 and antibody to GOR epitope (anti-GOR) to screen serum. A GOR epitope was isolated from the plasma of a chimpanzee infected with a human non-A, non-B hepatitis agent. An ELISA was developed for anti-GOR and circulating HCV RNA can be detected by polymerase chain reaction (PCR) in most patients seropositive for anti-GOR but negative for anti-c1004). The GOR epitope contains part of the amino acids from the core protein of HCV RNA5). We detected HCV RNA by PCR in 20 of 25 non-A, non-B hepatitis patients with anti-GOR alone6). Therfore, the combined use of anti-c100 and anti-GOR reveals more accurately the prevalence of HCV infection.

The prevalence of anti-c100 in blood donors has been reported in Europe7,8,9), the US10) and Japan11,12). The extent HCV infection in the general populations is unclear because the age of the donors ranged from 15 to 64 years. We surveyed for HCV infection not only in donors but also in children (under 15 years) and aged persons (over 65 years) in order to better determine the prevalence of HCV infection. The general populations of Fukuoka and Okinawa, Japan were surveyed, using anti-c100 and anti-GOR tests.

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MATERIALS AND METHODS

Studied population
A total of 4,624 serum samples were available, including 2,835 collected in Fukuoka Prefecture and 1,789 in Okinawa, Japan in 1990. In Fukuoka Prefecture, the serum samples were taken from 2,031 volunteer blood donors (15-64 years old), 512 outpatients of one of the satellite hospitals (0-14 years) and 292 people who came to another hospital (over 65 years) for a through physical check-up. Most patients under 14 years were being seen for the common cold. In Okinawa Prefecture, we offered free health examinations and announced them by distributing written notices to households. Samples were taken from the persons who came to be examined. Okinawa is located in the subtropical zone about 1,000 km south of the main islands of Japan and were controlled by the US from 1945-72. History of episodes of blood transfusion and surgery were obtained from 588 subjects whose serum samples were tested for anti-HCV and anti-GOR, 430 from Fukuoka and 158 from Okinawa in over the age of 50. All serum samples were stored at -20°C until tested.

Methods
Anti-c100 was tested by ELISA (Ortho HCV, Ortho Diagnostics, Raritan, New Jersey, USA) as described in detail by Kuo et al.25. The specimens considered reactive by the Ortho-HCV ELISA test in duplicate tests were retested using a supplementary test; recombinant immunoblot assay (RIBA; Ortho Diagnostics, Raritan, New Jersey, USA). This procedure involves the use of three recombinant antigens; c100-3, 5-1-1, and human superoxide dismutase (SOD) which is present as a control to detect the presence of antibodies against SOD and which are not specific for the HCV-related portions of the recombinant HCV antigens. The subjects reactive by the RIBA test were considered positive for anti-c100.

Anti-GOR was tested by ELISA (Institute of Immunology, Tokyo, Japan). This indirect assay involves use of an epitope with the amino acid sequence GRRGQKAKSNPNRPL (GOR epitope) derived from a cDNA clone (GOR 47-1), as described in detail by Mishiro et al.49. Neutralization test of anti-GOR was performed. The reactivities of antibody were determined by adsorbing the specimens with the GOR antigen and then by testing the adsorbed and unadsorbed sera for antibody by commercial ELISA procedure. A 50% reduction in counts between the unadsorbed sera was considered to be a positive result for antibody.

Serum alanine aminotransferase (ALT) over 36 IU/L was regarded as being abnormally high.

Statistical Analysis
The chi-square test was used for the statistical analysis. A p value of less than 0.05 was considered to be significant.

RESULTS
The prevalence of anti-c100 was 2.1 percent in Fukuoka and 0.7 percent in Okinawa. The preva-
Infection in the Japanese Population

The prevalence of anti-GOR was 2.6 percent in Fukuoka. No anti-GOR-positive subjects were identified in Okinawa. Age-specific prevalences of HCV infection (anti-c100 and/or anti-GOR) in Fukuoka and Okinawa are summarized in Table 1. Data for Fukuoka and Okinawa are presented separately because the area surveyed in Fukuoka showed a pattern of HCV infection distinctly different from that found in Okinawa.

The overall rate of HCV infection was 3.7 percent in Fukuoka, significantly (p < 0.001) higher than the 0.7 percent in Okinawa. In Fukuoka, the prevalence of HCV infection was lowest (0.6 percent) in the 0-9 age group and increased with age. The prevalence of HCV infection was highest (11.5 percent) in persons over 70. In Okinawa, we found no one with HCV infection in the 0-9, 10-19, or 30-39 age groups. The prevalence of HCV infection was highest (1.9 percent) in the 40-49 age group. The prevalence of HCV infection in all age groups in Fukuoka was higher than that in Okinawa. In the 60-69 and over 70 age groups, the prevalence of HCV infection was significantly higher in Fukuoka than in Okinawa (p < 0.001). There was no significant difference in the distribution of HCV infection between the sexes in either Fukuoka or Okinawa.

Table 2. The episodes of blood transfusion and surgery in the general population of Fukuoka and Okinawa.

| District | No. surveyed | blood transfusion + (%) | Operation + (%) |
|----------|--------------|-------------------------|-----------------|
| Fukuoka  | 430          | 80(18.6)***             | 244(56.7)***    |
| Okinawa  | 158          | 11(7.0)***              | 61(38.6)***     |

*** p < 0.001

Table 3. Prevalence of liver dysfunction in subjects with hepatitis C virus infection in Fukuoka.

| anti-c100 | anti-GOR | No. subjects | liver dysfunction + (%) |
|-----------|----------|--------------|-------------------------|
| +         | +        | 27           | 9(33.3)*                |
| +         | −        | 48           | 6(12.5)*                |
| −         | +        | 41           | 5(12.2)                 |
| Total     |          | 116          | 20(17.2)                |

anti-c100: antibody to c100 protein
anti-GOR: antibody to GOR epitope
* p < 0.05

DISCUSSION

Our data (3.7 percent in Fukuoka and 0.7 percent in Okinawa) represent reliable rate data on HCV infection in two general populations. We previously reported that the prevalence of anti-c100 was 1.3 percent among mentally retarded patients in Okinawa. This study confirmed that the prevalence of HCV infection in Okinawa is low, although this area is a highly endemic area for hepatitis B virus (HBV) (hepatitis B surface antigen 7.5 percent, antibody to hepatitis B core antigen 65.5 percent in 1979-1981) and human T lymphotropic virus type I (HTLV-I) (antibody to HTLV-I 15.5-17.1 percent in 1980-84) (15, 16). In Ehime prefecture, which is also an endemic area for HBV and HTLV-I infections, the prevalence of anti-c100 was not significantly different from that of Tokyo blood donors. These data suggest that the prevalence of HCV infection can not be related to HBV and HTLV-I prevalence.

The prevalence of HCV infection was significantly higher in Fukuoka than in Okinawa, especially in persons over 60 years of age. This high seropositivity for HCV in the general population could account for the high prevalence of anti-C100 found in patients with chronic liver disease in Fukuoka. HCV is spread via infected blood and through sexual intercourse. Perinatal transmission from mother to child is the most important route in the case of HBV and HTLV-I infections, the prevalence of anti-c100 was not significantly different from that of Tokyo blood donors. These data suggest that the prevalence of HCV infection can not be related to HBV and HTLV-I prevalence.

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Elderly persons in Fukuoka had had more episodes of blood transfusion and surgery than had those in Okinawa. This would suggest that the main route of HCV infection in Fukuoka is medical treatment in-
cluding blood transfusion, and would also explain the low prevalence of HCV infection in Okinawa. Although the history of intravenous drug use, tattooing and homosexual behavior were not completely investigated in the present study, no subjects with HCV infection in the over 60 age group had these histories.

It seems possible that the difference in prevalence of HCV infection between Fukuoka and Okinawa may be due to the historical difference in the system of blood supply. Before 1952, blood for transfusion was supplied by relatives and friends, but, since 1953, by paid donors at one of three commercial blood banks in Fukuoka. In 1962 a blood center was established to obtain blood for transfusion from volunteer donors in Fukuoka. Because many transfused patients complained of posttransfusion hepatitis, the Japanese government decided that blood for transfusion must be collected from volunteer donors in 1964. In 1969 all commercial blood banks had discontinued the collection of blood from paid donors and all blood for transfusion has been supplied by volunteer donors at blood centers since 1970. The paid donors belonged to the lower socioeconomic class, and the prevalence of HCV infection is known to be associated with socioeconomic factors. On the other hand, the blood for transfusion was supplied by a patient’s relatives and friends before a blood center for volunteer donors was established in 1967 in Okinawa. Therefore, blood for transfusion by paid donors has never been used in Okinawa. This may be one reason for the difference in the prevalence of HCV infection between the two areas studied.

It has been reported that 20 (15.7 percent) of 127 anti-c100-positive patients undergoing hemodialysis had liver dysfunction. There were few HCV infected persons found with liver dysfunction in the general population. The finding that the prevalence of liver dysfunction is low in the general population suggests that a lower immune response due to hemodialysis is not a critical factor in the occurrence of HCV-related liver dysfunction.

It is interesting that liver dysfunction is significantly more prevalent in persons with a concurrence of anti-c100 and anti-GOR than in persons with anti-c100 or anti-GOR alone. This may relate to the fact that HCV RNA can be detected by PCR in all non-A, non-B hepatitis patients with concurrence of anti-c100 and anti-GOR. Consequently, it may be that anti-c100 alone or anti-GOR alone can be detected in only part of the subjects with past HCV infection.

We concluded from this survey that HCV is most often spread by medical treatment, especially blood transfusion, in Fukuoka. Paid donor blood for transfusion plays an important role in HCV infection in Japan.

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