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Sero-epidemiologic study of hepatitis C virus infection in Fukuoka, Japan

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Abstract: We conducted an epidemiological study of 509 residents of H town, Fukuoka, Japan, to investigate the high mortality rate from liver disease. Antibodies to hepatitis C virus (HCV) (anti-HCV) were detected in 120 residents (23.6%); HCV RNA in 91 (17.9%), and hepatitis B surface antigen (HBsAg) in 13 (2.6%). Multivariate logistic regression analyses showed that presence of anti-HCV, male gender, and history of liver disease were associated with the presence of liver dysfunction, and that age of more than 40 years and a particular district were associated with the presence of anti-HCV. HCV RNA was more frequently detected in anti-HCV-positive men than women (41, or 85.4% versus 50, or 69.4%) ($P < 0.05$). The incidence of liver dysfunction was significantly higher in HCV RNA-positive men than women (32, or 66.7% versus 22, or 30.6%) ($P < 0.05$). These findings suggest that: (1) HCV was correlated with the high mortality rate from liver diseases, (2) there were district-related differences in the incidence of HCV, and (3) the lower frequency of elimination of HCV from men may explain why they showed a high mortality from liver disease.

Key words: hepatitis C virus (HCV), HCV hyper-endemic area, HCV RNA

Introduction

The hepatitis C virus (HCV) cDNA clone was isolated[1] in 1989; screening for hepatitis then became possible with tests for widespread HCV antibody testing.[2,3] Such testing revealed that HCV was hyper-endemic in several areas of Japan.[4–6] H town, in Fukuoka Prefecture in northern Kyushu, Japan, has four areas, designated S, K, H, and M, with populations of 2375, 2559, 3389, and 1476, respectively. The mortality from liver diseases [hepatocellular carcinoma (HCC) and hepatic failure] in H town between 1984 and 1988 was reported to be about three times higher than the national average in Japan (74.7% versus 24.1%).[7] It was also found that mortality from liver disease was much higher in men (74.5 per 100000 population) than in women (6.9 per 100000 population) in this town.[7] To identify the risk that contributed to the high prevalence of liver disease and to explain the gender-related differences in mortality from liver disease, we conducted a sero-epidemiological study of the inhabitants of H town in 1990.

Subjects and methods

Subjects

In 1990, of the total 9799 inhabitants, 10% of the 7389 inhabitants more than 20 years old (739 people) were randomly selected as follows. The names of the residents (as they appeared on their resident card) were arranged in order according to the Japanese phonetic syllabary. Then every tenth resident was selected. As a result, 509 subjects (6.9% of H town residents) gave their informed consent to participate in the study. All of the subjects in this study were thus selected randomly. The mean age of men and women did not differ significantly (217 men; age, 49 ± 16 years, range 20–91 years; 292 women; age, 54 ± 17 years, range, 20–94 years) (Table 1).

This town has experienced little migratory activity. Agriculture is the main industry, and the life style and educational standards of the residents correspond to the national averages. None of the inhabitants reported being homosexual or being a carrier of HIV-1 (human

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Table 1. Age-specific incidence of liver dysfunction and prevalance of viral markers

Age (years)	Number of subjects (men/women)	Liver dysfunction (%)	Anti-HCV (%)	HCV RNA (%)	HBsAg (%)	Anti-HBc (%)
20–29	49 (26/23)	6 (12.2)	2 (4.1)	2 (4.1)	0	11 (22.4)
30–39	90 (47/43)	16 (17.8)	10 (11.1)	8 (8.9)	3 (3.3)	26 (28.9)
40–49	96 (44/52)	24 (25.0)	17 (17.8)	14 (14.6)	5 (5.2)	36 (37.5)
50–59	94 (35/59)	25 (26.6)	31 (33.0)	22 (23.4)	4 (4.3)	53 (56.4)
60–69	93 (38/55)	22 (23.7)	31 (33.3)	22 (23.7)	1 (1.1)	55 (59.1)
70–79	57 (21/36)	11 (19.3)	22 (38.6)	18 (31.6)	0	32 (56.1)
80–	30 (6/24)	1 (3.3)	7 (23.3)	5 (16.7)	0	21 (70.0)
Total	509 (217/292)	105 (20.6)	120 (23.6)	91 (17.9)	13 (2.6)	234 (46.0)

Liver dysfunction was defined as AST $>$ 40 IU/l and/or ALT $>$ 30 IU/l
AST, Aspartate aminotransferase; ALT, alanine aminotransferase; anti-HCV, antibody to hepatitis C virus; HBsAg, hepatitis B surface antigen; anti-HBc, antibody to HBV core antigen

immunodeficiency virus-1). The traditional Japanese healing practice known as vacuuming[5] is not used as a conventional folk remedy in this town. (Vacuuming is so-called "Suidama" therapy, which is popular as a folk remedy in some area of Japan. It is claimed to relieve muscle stiffness by the removal of blood. Firstly, the skin is cut over the muscle with a nonsterile knife. Secondly, the wound is then covered by a warming bottle so that blood is drawn from the wound into the bottle. This therapy is usually performed by a family.) All participants were interviewed to determine possible risk factors (Tables 2 and 3). The study was approved by the Ethics Committee of Kurume University Hospital.

Serological assay

Sera were collected from all the participants, and conventional liver function tests were performed: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined. The presence of liver dysfunction was defined as a serum level of AST exceeding 40 IU/l and/or ALT exceeding 30 IU/l. Hepatitis B surface antigen (HBsAg) and antibody to hepatitis B (HB) core antigen (anti-HBc) were assayed by enzyme immuno-assay (EIA) (Mizuho Medy, Tosu, Japan), and anti-HCV by second-generation passive hemagglutination assay (HCV PHA second generation, Dainabot, Tokyo, Japan), and all these results were confirmed by a second-generation recombinant immunoblot assay (RIBA II) (Ortho Diagnostic System, Tokyo, Japan).

Detection of HCV RNA by RT-PCR

All subjects who were anti-HCV-positive were tested for the presence of serum HCV RNA, which was detected by reverse transcriptase-nested polymerase chain reaction (RT-nested PCR) using primers based on the sequences of the 5′UTR (untranslated region) of the HCV genome, as described previously.[8]

Statistical analysis

Values are expressed as means ± SD. Statistical analyses were performed using the χ^2 test with Yate's correction, and two-tailed Fisher's exact test. Multivariate analysis was performed by the logistic regression method. The SAS (statistical analysis system) computer program (SAS Institute Japan Ltd, Tokyo, Japan) was used for all analyses. A probability value of less than 0.05 on two-tailed analyses was considered significant.

Results

Age-specific incidence of liver dysfunction and prevalence of viral markers (Table 1)

Of the 509 participants, 105 (20.6%) had liver dysfunction. Anti-HCV was detected in 120 (23.6%) of the subjects, HCV RNA in 91 (17.9%), HBsAg in 13 (2.6%), and anti-HBc in 234 subjects (46.0%). The incidence of liver dysfunction and the prevalences of anti-HCV, HCV RNA, and anti-HBc increased gradually with age. The prevalence of HBsAg did not increase with age.

Univariate and multivariate analyses of parameters that influence liver dysfunction (Table 2)

Univariate analyses revealed correlations between liver dysfunction and 9 of 11 parameters related to the subjects' demographic characteristics and medical history ($P < 0.05$). These 9 factors were evaluated by multivariate analysis. Multivariate logistic regression analysis of parameters that were marginally related to liver dysfunction showed that anti-HCV, gender, and past history of liver disease were associated with liver dysfunction ($P = 0.0001$).

Table 2. Univariate and multivariate analyses of parameters that influence liver dysfunction

Parameter		Univariate analysis: No. of subjects with liver dysfunction/ total no. (%)	*P* Value
Age (years)	(CODE)		
20–39	(0)	22/139 (15.8)	
40–	(1)	83/370 (22.4)	0.1008
Sex	(CODE)		
Men	(0)	69/217 (31.8)	
Women	(1)	36/292 (12.3)	0.0001
History of liver disease	(CODE)		
No	(0)	59/416 (14.2)	
Yes	(1)	46/92 (50.0)	0.0001
History of icterus	(CODE)		
No	(0)	85/470 (18.1)	
Yes	(1)	20/38 (52.6)	0.0001
History of blood transfusion	(CODE)		
No	(0)	89/466 (19.1)	
Yes	(1)	16/42 (38.1)	0.0040
Family history of liver disease	(CODE)		
No	(0)	75/359 (20.9)	
Yes	(1)	30/149 (20.1)	0.8480
Heavy alcohol consumption	(CODE)		
No	(0)	86/455 (18.9)	
Yes	(1)	14/41 (43.9)	0.0001
District	(CODE)		
S	(1)	33/131 (25.2)	
K	(2)	30/136 (22.1)	
H	(3)	34/158 (21.5)	
M	(4)	8/84 (9.5)	0.0410
HBsAg	(CODE)		
Negative	(0)	99/496 (20.0)	
Positive	(1)	6/13 (46.2)	0.0327
Anti-HBc	(CODE)		
Negative	(0)	42/275 (15.3)	
Positive	(1)	63/234 (26.9)	0.0010
Anti-HCV	(CODE)		
Negative	(0)	49/389 (12.6)	
Positive	(1)	56/120 (46.7)	0.0001

Parameter	Multivariate analysis: Parameter estimate	*P* Value	Odds ratio (95% CI)
Anti-HCV	1.5287	0.0001	4.16 (3.55–4.77)
Sex	1.2967	0.0001	3.53 (2.81–4.24)
History of liver disease	1.0226	0.0001	2.78 (2.17–3.39)

Liver dysfunction was defined as AST > 40 IU/l and/or ALT > 30 IU/l; history of liver disease was defined as formerly pointed out liver disfunction. Heavy alcohol consumption was defined as a daily intake of 75 g ethanol per day for more than 10 years

AST, Aspartate aminotransferase; ALT, alanine aminotransferase; HBsAg, hepatitis B virus surface antigen; Anti-HBc, antibody to HBV core antigen; anti-HCV, antibody to hepatitis C virus; CI, confidence interval

Univariate and multivariate analyses of parameters that influence anti-HCV (Table 3)

To determine the risk factors for anti-HCV positivity, univariate analyses of correlations between anti-HCV and 13 factors was carried out; significant differences ($P < 0.05$) were seen for 5 of these factors. Multivariate analysis showed that age and the district in which the participant resided ($P = 0.0306$, $P = 0.0025$, respectively) were significantly associated with anti-HCV positivity.

Distribution of hepatitis viral markers and incidence of liver dysfunction by gender (Table 4)

The prevalence of HBsAg, anti-HBc, and anti-HCV did not differ significantly between men and women. HCV RNA was more frequently detected in the anti-HCV-positive men (41/48; 85.4%) than in the anti-HCV-positive women (50/72; 69.4%) ($P < 0.05$). The incidence of liver dysfunction was significantly higher in the HCV RNA-positive men (32/48; 66.7%) than in the HCV RNA-positive women (22/72; 30.6%) ($P < 0.05$). The incidence of liver dysfunction in HCV RNA-positive subjects who were not heavy drinkers was also higher in men (26/32; 81.3%) than in women (22/50; 44.0%) ($P < 0.05$).

Discussion

To investigate the high mortality rate[7] from liver disease and the gender-related differences in the mortality rate in H town, we conducted a sero-epidemiologic study of about 10% of its adult inhabitants. We found that the prevalence of HBV-related markers (HBsAg and anti-HBc; 1.5% and 40.1%, respectively) did not differ significantly from national averages. However, the prevalence of anti-HCV among the participants in this study exceeded the prevalence reported among blood donors in Japan[9] and in the United States.[10] Multivariate statistical analysis also demonstrated that positivity for anti-HCV and a history of liver disease were associated with liver dysfunction. These findings suggest that HCV infection is a main causal agent of liver disease, and that the high prevalence of HCV infection may be related to the high mortality rate[7] from liver disease in H town. Multivariate statistical analysis also demonstrated that male gender was associated with liver dysfunction. Although the prevalence of virus markers (HBsAg, anti-HBc, and anti-HCV) did not differ significantly between the sexes, HCV RNA was more frequently detected among the anti-HCV-positive men than among the anti-HCV positive women. The incidence of liver dysfunction was significantly higher in the HCV RNA-positive men than in HCV RNA-positive women, even when heavy drinkers were excluded from the analysis.

Table 3. Univariate and multivariate analyses of parameters that influence anti-HCV

Parameter		Univariate analysis: No. of anti-HCV positive/total no. (%)	*P* Value
Age (years)	(CODE)		
20–39	(0)	12/139 (8.6)	
40–	(1)	108/370 (29.2)	0.0001
Sex	(CODE)		
Men	(0)	48/217 (22.1)	
Women	(1)	72/292 (24.7)	0.5050
History of liver disease	(CODE)		
No	(0)	62/416 (14.9)	
Yes	(1)	58/92 (63.0)	0.0001
History of blood transfusion	(CODE)		
No	(0)	104/466 (22.3)	
Yes	(1)	16/42 (38.1)	0.0210
Family history of liver disease	(CODE)		
No	(0)	77/359 (21.5)	
Yes	(1)	43/149 (28.9)	0.0730
Heavy alcohol consumption	(CODE)		
No	(0)	105/455 (23.1)	
Yes	(1)	12/41 (29.3)	0.3750
History of acupuncture	(CODE)		
No	(0)	67/281 (23.8)	
Yes	(1)	29/126 (23.0)	0.8560
History of employment in a health-care profession	(CODE)		
No	(0)	89/374 (23.8)	
Yes	(1)	7/25 (28.0)	0.6340
History of intravenous drug abuse	(CODE)		
No	(0)	94/397 (23.7)	
Yes	(1)	2/5 (40.0)	0.3435
History of tattooing	(CODE)		
No	(0)	94/402 (23.4)	
Yes	(1)	2/3 (66.7)	0.1412
District	(CODE)		
S	(1)	50/131 (38.2)	
K	(2)	35/136 (25.7)	
H	(3)	22/158 (13.9)	
M	(4)	13/84 (15.5)	0.0001
HBsAg	(CODE)		
Negative	(0)	117/496 (23.6)	
Positive	(1)	3/13 (23.1)	0.9660
Anti-HBc	(CODE)		
Negative	(0)	40/275 (14.6)	
Positive	(1)	80/234 (34.2)	0.0001

Parameter	Multivariate analysis: Parameter estimate	*P* Value
Age (years)	0.0265	0.0306
Area	0.5087	0.0025

Liver dysfunction was defined as AST > 40 IU/l and/or ALT > 30 IU/l; history of liver disease was defined as formerly pointed out liver disfunction. Heavy alcohol consumption was defined as a daily intake of 75 g ethanol per day for more than 10 years

AST, Aspartate aminotransferase; ALT, alanine aminotransferase; HBsAg, hepatitis B virus surface antigen; anti-HBc, antibody to HBV core antigen; anti-HCV, antibody to hepatitis C virus

These observations led us to hypothesize that HCV is less likely to be eliminated from serum in men, and that HCV-related liver injury is more severe in men than in women. A substantial proportion of patients with chronic hepatitis develop cirrhosis and HCC.[11,12] According to statistical surveys conducted in Japan, approximately 80% of patients with HCC are positive for HCV.[13] These factors may explain why the mortality rate from liver disease is higher in men than in women.

We conducted a multivariate analysis of the possible risk factors for HCV infection to identify the routes of HCV infection in H town. As HCV is typically transmitted via the blood, known risk factors include blood transfusions and/or the use of parenteral drugs.[14–16] Person-to-person transmission of HCV has also been suggested.[17–20] Previous reports from other areas hyperendemic for HCV in Japan indicate an increased risk of HCV infection related to various types of medical treatments, such as folk remedies[5] (acupuncture and/or vacuuming[5]), and the use of non-sterilized medical instruments by a family physician,[5,6] but have not shown familial transmission of HCV to be of importance.[5,6] In the present study, intravenous drug abuse, blood transfusion, acupuncture, and family history of liver disease were not associated with anti-HCV positivity, but the district of residence was associated with anti-HCV positivity. Interviews with many of the residents and nurses revealed that a family physician (whose clinic is in S area) had often used non-sterilized syringes and needles. The spread of HCV infection, which had a

Table 4. Distribution of hepatitis virus markers and incidence of liver dysfunction by gender

Parameter	Men (%) (n = 217)	Women (%) (n = 292)	Total (%) (n = 509)
HBsAg	7 (3.2)	6 (2.1)	13 (2.6)
Anti-HBc	100 (46.1)	134 (45.9)	234 (46.0)
Anti-HCV	48 (22.1)	72 (24.7)	120 (23.6)
Parameter	**Men with anti-HCV (%) (n = 48)**	**Women with anti HCV (%) (n = 72)**	**Total (%) (n = 120)**
HCV RNA	41 (85.4)	50 (69.4)*	91 (75.8)
HCV RNA with liver disfunction	32 (66.7)	22 (30.6)*	54 (45.0)

* $P < 0.05$

Liver dysfunction was defined as AST (>40 IU/l) and/or ALT (>30 IU/l)

AST, Aspartate aminotransferase; ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; anti-HBc, antibody to HB core antigen; anti-HCV, antibody to HCV

particularly high rate in S area, may have been via this family physician. Multivariate analysis also demonstrated a high prevalence of anti-HCV among subjects aged more than 40 years, suggesting that HCV was spread by horizontal transmission via the contaminated syringes and needles.

HCV transmission by blood transfusion now occurs only rarely. Other measures to counteract known risk factors for HCV transmission, such as the use of disposable medical supplies, are also likely to decrease HCV infection in H town. The success of these current measures, however, remains uncertain, because it is conceivable that other HCV transmission routes exist in an area with a high anti-HCV incidence. To answer these questions and to evaluate patients with HCV-induced liver disease in more detail, a follow-up study is currently being conducted in H town.

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