Prevalence of Anti-Hepatitis C antibodies in a Rural Community without High Mortality from Liver Disease in Niigata Prefecture

Kazunori Kayaba 1, Masahiro Igarashi 1, Hiroaki Okamoto 2, and Fumio Tsuda 3

The prevalence of hepatitis C virus (HCV) infection and factors relating to the HCV transmission were evaluated in a community without high mortality from chronic liver disease in Niigata prefecture. A total of 2,231 subjects were examined to detect anti-HCV core antibodies by enzyme-linked immunosorbent assay with synthetic peptides CP14 and CP9. The prevalence was 1.66% (95% CI; 1.17% to 2.29%) and tended to increase with age. The values were lower than those reported from districts with hepatic disease endemic. Histories of blood transfusion (relative risk (RR) 5.51; 95% CI 2.90 to 10.48) and surgery with hospital admission (RR 4.43; 95% CI 2.04 to 9.65) were significantly associated with the anti-HCV core antibodies positive. Multiple logistic analysis corroborated independency of these factors. Among 188 subjects who experienced surgery and/or blood transfusion after 1990, only one (0.5%) had HCV infection. By contrast, 8 (3.5%) were positive in subjects who experienced first acupuncture therapy after 1990. The acupuncture therapy in alternative medicine could be still related to the HCV transmission. J Epidemiol, 1998; 8: 250-255.

Hepatitis C virus (HCV) is the first leading cause of chronic liver disease in Japan 9. Chronic hepatitis is a major preceding state for the cancer. Liver cancer is the third leading cause of deaths due to cancer in Japan 9. Some clinical studies showed that a part of HCV was responsive to interferon therapy 9, and that a glycyrrhizin was effective in preventing liver carcinogenesis 4. Therefore the community-based detection of non-symptomatic HCV carrier could be efficacious for preventing the liver cancer death 9.

Estimating the prevalence of the HCV carrier in populations and detecting factors relating to the HCV transmission are essential for the community-based control of the HCV infection. Serological methods for detecting anti-HCV antibodies have been available since 1989 9. The methods were introduced mainly to screenings in blood donors 6,7, and in some communities with high prevalence of hepatitis or hepatic cancer 8,10. In those populations, anti-HCV antibodies were detected in 20-30% of residents. On the other hand, few studies reported the prevalence of the HCV infection in the community without high incidence of hepatic diseases, which seems to represent general Japanese population. Such a data contributes to a nationwide prevention for HCV hepatitis and hepatic cancer death.

We, therefore, conducted a population-based survey for evaluating the prevalence of HCV infection and factors relating to the HCV infection in a community in Niigata prefecture.

STUDY POPULATION AND METHODS

The subjects were participants for the mass screening examinations in accordance with the health and medical service law for aged. The participants are residents in Y Town, Niigata, an agriculture fields in which the standardized mortality ratio for chronic hepatitis and liver cirrhosis from 1989 to 1993 was 0.12 in men and 0.22 in women 9. The study was approved by the local government in Y. Of 2,583 residents eligible for the
law in Y, 2,569 (99.5%) were present at the examinations in September 1995 and June and July 1996. After interviewing the histories of liver diseases and hepatitis virus infection, the informed consent was performed. Finally, 2,231 examinees agreed to participate in this study. 1,544 (69.2%) were women. Final response rate was 86.4%.

Trained interviews assessed histories of alcohol drinking and family history of liver diseases. Informations on blood transfusion, surgery with hospital admission, acupuncture therapy, and the first year in which subjects experienced them were obtained.

The anti-HCV core antibodies were detected by commercial kit [SMITEST (HCV Core Ab ELISA), Sumitomo Metal Industries, Tokyo, Japan] with synthetic peptides CP14 and CP9 predicted from the sequence of the HCV C-region genome. Absorbance at 450 nm ≥ 1.500 was considered as positive. Blood specimens for the antibodies detection were collected in individual siliconized glass tubes.

Serum total protein and albumin, titer of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (Alp), γ-glutamyl transpeptidase (γ-GTP) and Cholinesterase (ChE), and platelet count were determined at the chemical laboratory of Yukiguni Yamato General Hospital. All measurements were performed by an autoanalyzer Hitachi 7170 (Hitachi, Hitachi, Japan). Values of 90 percentile or more were regarded as abnormal in categorical analysis.

Statistical analysis was performed using the SPSS release 6.1J for Windows (SPSS Inc., Chicago, Illinois). 95% confidence interval of the prevalence of the anti-HCV antibodies was estimated using the Poisson distribution for observed numbers. Man-Whitney U test was performed for continuous variables. For a comparison of categorical data, Fisher’s exact probability test was used. We observed a ratio of the prevalence of the anti-HCV antibodies among subjects exposed to the prevalence among those unexposed as a relative risk (RR), and its 95% confidence interval (95% CI) was estimated. To assess the influence of each variable, a logistic regression model was performed by using significant variables in the univariate analyses. A significant difference in two-tailed test was defined at p < 0.05.

**RESULTS**

Table 1 shows differences between participants and non-participants in age, sex, the histories, and results of the blood tests. Comparing to participants, non-participants were older and had higher prevalence of men or past history of HCV infection. Blood tests showed no significant difference except for the prevalence of low albuminemia. Among the examinees with the history of HCV infection, there was not statistically significant difference in results of blood tests between participants and non-participants.

In Table 2, overall prevalence of anti-HCV antibodies positive was 1.66%. Its 95% confidence interval was estimated to be 1.17% to 2.29%. The prevalence was slightly higher in women (women 31/1544 = 2.01% (95% CI; 1.36% to 2.85%),

### Table 1. Comparison of selected characteristics between participants and non-participants among total examinees.

|                      | Participants | Non-participants | p-value |
|----------------------|--------------|------------------|---------|
| **Total**            | 2231         | 338              |         |
| Age (Mean±SD)        | 53.8±14.3    | 56.0±14.5        | <0.01   |
| Women                | 1544 (69.2)  | 209 (61.8)       |         |
| Men                  | 687 (30.8)   | 129 (38.2)       | <0.01   |
| Positive medical history |            |                  |         |
| Liver disease        | 242 (10.8)   | 29 ( 8.6)        |         |
| Hepatitis C virus infection | 6 ( 0.3)   | 8 ( 2.4)        | <0.0001 |
| Hepatitis B virus infection | 26 ( 1.2) | 7 ( 2.1)        |         |
| Abnormal in blood test |             |                  |         |
| Serum total protein (<6.8 gram/deciliter) | 216 (9.8) | 35 (10.6) |         |
| Serum albumin (<3.0 gram/deciliter) | 182 (8.2) | 43 (13.1) | <0.01   |
| AST* (>34 IU/liter)  | 208 (9.3)    | 41 (12.2)        |         |
| ALT** (>36 IU/liter) | 192 (8.6)    | 38 (11.3)        |         |
| γ-GTP*** (>47 IU/liter) | 224 (10.0) | 37 (11.0)       |         |
| Alkaline phosphatase (>315 IU/liter) | 217 (9.7) | 36 (10.7)       |         |
| Cholinesterase (<231 IU/liter) | 240 (10.8) | 37 (11.0)       |         |
| Platelet (<16 × 10⁹/mm³) | 186 (8.3) | 30 (8.9)        |         |

Each set of values is number and percentage (%), if not otherwise specified.

* aspartate aminotransferase ** alanine aminotransferase *** γ-glutamyl transferase
men 6/687 = 0.87% (0.32% to 1.90%), not statistically significant]. Subjects aged 50 years or more had higher prevalence of positive anti-HCV antibodies (≥50 years 31/1372 = 2.26%, <50 years 6/859 = 0.70%, RR 3.23 (1.36 - 7.72)).

Histories of blood transfusion and surgery with hospital admission were significantly higher prevalent in subjects with anti-HCV antibodies than in those without the antibodies (Table 3). Relative risk (95% CI) was 5.51 (2.90 to 10.48) in blood transfusion and 4.43 (2.04 to 9.65) in surgery.

Table 4 shows the prevalence of anti-HCV antibodies by blood transfusion, surgery, and an acupuncture therapy stratified by the year in which subjects experienced them first. The screening test for anti-HCV antibodies has been introduced to the Japan Red Cross blood transfusion centers and clinical settings since 1989. Among 188 subjects who had the blood transfusion and/or the surgery first after 1990, only one was HCV-positive. Contrarily, 8 (3.5%) were positive in subjects who experienced first acupuncture therapy after 1990. All of the 8 subjects were 50 years old or more. Between the acupuncture subjects aged 50 years or more and those less than 50 years, there was borderline difference in the proportion of subjects who experienced the acupuncture therapy first after 1990 (≥ 50 years 156/1373 = 11.4%, < 50 years 74/859 = 8.6%, p=0.06).

Table 5 shows results of the multiple logistic regression analysis in which age and significant variables in univariate analysis were adjusted for simultaneously to estimate odds ratio for positive anti-HCV antibodies. The age of 50 years or

| Table 2. Prevalence (%) of positive anti-HCV antibodies* by age. |
|---|---|---|---|---|
| Age (years) | No. of subjects | No. of positive | % | (95% CI) |
| 20-29 | 142 | 1 | 0.70 | (0.02-3.92) |
| 30-39 | 283 | 2 | 0.71 | (0.09-2.55) |
| 40-49 | 434 | 3 | 0.69 | (0.14-2.02) |
| 50-59 | 377 | 7 | 1.86 | (0.75-3.83) |
| 60-69 | 779 | 20 | 2.57 | (1.57-3.97) |
| 70+ | 216 | 4 | 1.85 | (0.50-4.74) |
| Total | 2,231 | 37 | 1.66 | (1.17-2.29) |

* Cut off value ≥1.500 at 450 nm

| Table 3. Relative risks (RRs) for positive anti-HCV antibodies in factors relating to HCV infection. |
|---|---|---|---|---|
| HCV | Positive N (%) | Negative N (%) | RR (95% CI) |
| History of liver diseases | yes | 7 (0.3) | 235 (10.5) | 1.92 (0.85-4.32) |
| no | 30 (1.3) | 1959 (87.8) | | |
| Alcohol drinking | current drinker | 10 (0.4) | 652 (29.3) | 0.91 (0.44-1.87) |
| non-drinker | 26 (1.2) | 1538 (69.1) | | |
| Blood transfusion | yes | 15 (0.7) | 230 (10.3) | 5.51 (2.90-10.48) |
| no | 22 (1.0) | 1958 (88.0) | | |
| Surgery with hospital admission | yes | 29 (1.3) | 973 (43.7) | 4.43 (2.04-9.65) |
| no | 8 (0.4) | 1217 (54.6) | | |
| Acupuncture therapy | yes | 11 (0.5) | 536 (24.1) | 1.30 (0.65-2.61) |
| no | 26 (1.2) | 1654 (74.3) | | |
| Family history of liver disease | yes | 3 (0.1) | 169 (7.6) | 1.05 (0.33-3.40) |
| no | 34 (1.5) | 2020 (90.7) | | |
Table 4. Prevalence (%) of anti-HCV antibodies by blood transfusion, surgical operation, and acupuncture based on the year in which subjects experienced them first.

| The year of first experience | Blood transfusion | Surgery | Acupuncture |
|-----------------------------|-------------------|---------|-------------|
|                             | Positive / Total   | Positive / Total | Positive / Total   |
| Before 1959                 | 3/24 (12.5)       | 10/166 (6.0)   | 0/24 (0.0)       |
| 1960-69                     | 7/53 (13.2)       | 8/210 (3.8)    | 2/23 (8.7)       |
| 1970-79                     | 3/67 (4.5)        | 3/239 (1.3)    | 0/72 (0.0)       |
| 1980-89                     | 2/62 (3.2)        | 7/184 (3.8)    | 1/175 (0.6)      |
| After 1990                  | 0/20 (0.0)        | 1/187 (0.5)    | 8/230 (3.5)      |

Table 5. Odds ratios (ORs) of positive anti-HCV antibodies by multiple logistic model.

|                    | OR       | (95% CI) |
|--------------------|----------|----------|
| Age ≥ 50 years     | 2.50     | (1.03-6.10) |
| Blood transfusion  | 3.49     | (1.71-7.15) |
| Surgery with hospital admission | 2.89 | (1.25-6.69) |

over, blood transfusion, and surgery remained as significant factors. The significance of the odds ratios was essentially unchanged when the history of first acupuncture therapy in whole period or that after 1990 was included in the logistic model.

Proportions of participants having an abnormal value of serum albumin (18.9% in anti-HCV antibodies positive subjects vs. 8.1% in the antibodies negative subjects), AST (32.4% vs. 8.9%), ALT (24.3% vs. 8.3%), Alp (27.0% vs. 9.4%), ChE (27.0% vs. 10.5%), and platelet count (24.3% vs. 8.1%) were significantly larger in subjects with anti-HCV antibodies.

**DISCUSSION**

The prevalence of anti-HCV antibodies in healthy Japanese populations has been reported in blood donors (0.2-1.8% measured by second generation antibodies) \(^6\), female prostitutes (10.1%), and residents in the liver disease endemic area (20-30%) \(^8,11\). Few studies evaluated the prevalence of positive anti-HCV antibodies in the community without high mortality or morbidity from liver disease.

In some studies \(^6,17\) data from blood donors represented “normal value”. A nationwide prevalence of HCV carrier was estimated to be 1-2% by using data from screenings in blood donors \(^6,18\). However, the donors have been selected from all applicants by some blood tests of liver function even before the introduction of the anti-HCV antibodies detection for transfusion blood, and some healthy persons donate repeatedly. Therefore the prevalence of HCV carrier in blood donors could be lower than that in the whole community-residents which the donors belong to. Majority of Japanese population is not a resident in liver disease endemic area. There seems to be a lack of data from the majority of the population. Our results partly contribute to elimination of the lack.

The prevalence of the anti-HCV antibodies positive subject in this study was apparently lower than those in liver disease endemic areas, and was slightly lower than those in the area adjoining districts of liver disease endemic (2.3-2.7%) \(^9,10\).

Elder subjects in our study tended to have higher prevalence of the antibodies, and non-participants was older than participants. Eight of 14 persons (57.1%) having the history of HCV infection did not participated in this examination. Therefore our results could underestimate the prevalence of anti-HCV antibodies positive in this community. If all of the 8 persons was anti-HCV antibodies positive, the prevalence increases by approximately 0.3%. On the other hand, 4 of 6 participants who answered having the HCV infection did not have positive anti-HCV antibodies titer in this examination, and there was no significant difference in results of blood tests between participants and non-participants with the history of HCV infection. These results suggest that the underestimation could be less than 0.3%.

Additionally, the prevalence of anti-HCV antibodies among blood donors in Niigata prefecture was low (0.3%) \(^6\). The mortality ratio for liver diseases at Y area was low as stated above \(^12\). On the basis of above evidences, the prevalence of the anti-HCV antibodies in this study could be located at the lower range in the distribution of the antibodies positive rate in Japanese population.

The anti-HCV antibodies generally mean not only ongoing HCV infection but residual reaction to the past infection. However, determination of HCV-RNA by polymerase chain reaction corroborated high sensitivity and specificity of our method in detecting the ongoing infection \(^10\).

The most important problem in the HCV infection is its ability to induce chronic liver disease and hepatocellular carcinoma \(^1,3\). The average time to cirrhosis is 20 years, and the average time to hepatocellular carcinoma is 30 years \(^20\). In this study, 80% of subjects with anti-HCV antibodies were 50 years of age or more, and 75% of the subjects have never expe-
Prevalence of Anti-HCV Antibodies in a Community without Liver Disease Endemic

inced any surgery, blood transfusion or an acupuncture since 1990 (Table 4). Assuming that more than 10 years have passed since the majority of the anti-HCV antibodies positive subjects were exposed to HCV, our subjects with HCV could have high possibility of developing cirrhosis or hepatocellular carcinoma from now.

It is important for infection control to detect the transmission pathway. HCV is transmitted primarily through blood and through human body secretions. Our study showed that blood transfusion and surgery with hospital admission were significant factors relating to the anti-HCV antibodies positive result. However, the transmission of HCV through blood transfusions has been eliminated since the Japan Red Cross introduced universal screening of HCV in donated bloods in 1989.

Previous studies reported that the history of an acupuncture or some folk remedies were associated with positivity for anti-HCV antibodies. In this study, an acupuncture therapy was not statistically significant. But it seems to be still associated with positive anti-HCV antibodies in this community as shown in Table 4. All of the 8 persons with positive anti-HCV antibodies among subjects with an acupuncture therapy first after 1990 were 50 years old or more. This result can not be fully explained by the difference of proportion of subjects having the therapy first after 1990 in each age group. Further examination is required to elucidate the relation of the acupuncture therapy to the HCV infection.

Although some recommendations for the prevention of hepatitis virus infection in health care facilities were made, public health officers should keep attention to HCV transmission through alternative medical cares like an acupuncture therapy in the Y area.

In conclusion, this study revealed the prevalence of the anti-HCV positive resident in a community with low mortality from chronic liver disease in Niigata prefecture. The prevalence in this study could be higher than that in blood donors, and was lower than that in communities with liver disease endemic. The prevalence in our subjects was suggested to be at lower range in general Japanese population. Blood transfusion and surgery with hospital admission were significantly associated with anti-HCV antibodies positive. After introducing the prevention of HCV infection in medical care settings in 1989, some alternative medicine, such as an acupuncture therapy, could be related to the HCV transmission. Elderly subjects with HCV infection should be followed because of high risk of developing hepatocellular carcinoma from now.

**ACKNOWLEDGMENTS**

This study was supported by a grant-in-aid from the Foundation for the Development of the Community, Tochigi, Japan. Authors are indebted to public health nurses and officers of the Yamato Health Examination Center in Niigata prefecture for their assisting data collection; to Ms. Etsuko Imai for her measuring an anti-HCV antibodies titer; and to Professor Makoto Mayumi at the immunology division of Jichi Medical School for his scientific advice.

**REFERENCES**

1. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med, 1993;328:1797-1801.
2. Health and Welfare Statistics Association. National public health, its trend. Kosei-no-Shiho, 1996;43:47-80 (in Japanese).
3. Sharara AI, Hunt CM, Hamilton JD. Hepatitis C. Ann Intern Med, 1996;125:658-668.
4. Arase Y, Ikeda K, Murashima N, Chayama K, Tsubota A, Koida I, Suzuki Y, Saitoh S, Kobayashi M, Kumada H. The long term efficacy of glycyrrhizin in chronic hepatitis C patients. Cancer, 1997;79:1494-1500.
5. Kuo G, Choo Q-L, Alter HJ, Gitnick GL, Redecker AG, Purcell RH, Miyamura T, Dienstag JL, Alter MJ, Stevens CE, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science, 1989;244:362-364.
6. Yoshihara N, Oota Y, Okabe N, Akiba T. Epidemiology of hepatitis C virus infection -Japan, China, Tonga, Fiji, Philippine-. In: Tanaka K and Obara M, eds. Hepatitis C, HCV detection and interferon therapy. Medical View, 1994:108-112 (in Japanese).
7. Tanaka J, Moriya T, Sasaki F, Yoshizawa H, Nagakami H, Irie A, Mizui M. Prevalence of anti C100-3 and HBsAg in blood donors. Nihon Koshu Eisei Zasshi, 1993;40:540-546 (in Japanese).
8. Ishibashi M, Shinzawa H, Kuboki M, Tsuchida H, and Takahashi T. Prevalence of inhabitants with anti-hepatitis C virus antibody in an area following an acute hepatitis C epidemic : age- and area-related features. J Epidemiol, 1996;6:1-8.
9. Hidaka Y, Hiramatsu Y, Tsuda F. High prevalence of HCV infection in a town where high mortality from liver disease is observed. Nihon Koshu Eisei Zasshi, 1996;43:9-15 (in Japanese).
10. Watanabe Y, Machida K, Sato A, Ota S, Kiyosawa K, Survey for hepatitis in an isolated endemic area. Nihon Koshu Eisei Zasshi, 1996; 43: 989-996 (in Japanese).
11. Koyama T, Tsuda F, Ishikawa K, Onishi H, Tazawa M, Yoshizawa H, Sato S, Okamoto H. Antibodies to hepatitis C virus and elevated transaminase levels in a town of hyperendemicity in Iwate, Japan. J Gastroenterol Hepatol, 1997;12:67-72.
12. Niigata Ken Seijinnbyo Yobou Kyoukai. Standardized
13. Okamoto H, Munekata E, Tsuda F, Takahashi K, Yotsumoto S, Tanaka T, Tachibana K, Akahane Y, Sugai Y, Miyakawa Y, Mayumi M. Enzyme-linked immunosorbent assay for antibodies against the capsid protein of hepatitis C virus with a synthetic oligopeptide. Japan J Exp Med, 1990;60:223-233.

14. Nagayama R, Miyake K, Tsuda F, Okamoto H. IgM antibody to a hepatitis C virus core peptide (CP14) for monitoring activity of liver disease in patients with acute or chronic hepatitis C. J Med Virol, 1994;42:311-317.

15. Morris JA, Gardner MJ. Calculating confidence intervals for relative risks, odds ratios, and standardised ratios and rates. In; Gardner MJ and Altman DG, eds. Statistics with confidence - Confidence intervals and statistical guidelines, British Medical Journal, 1989:50-63.

16. Nakashima K, Kashiwagi S, Hayashi J, Urabe K, Minami K, Maeda Y. Prevalence of hepatitis C virus infection among female prostitutes in Fukuoka, Japan. J Gastroenterol, 1996;31:664-668.

17. Simo R, Hernandez C, Genesca J, Jardi R, Mesa J. High prevalence of hepatitis C virus infection in diabetic patients. Diabetes Care, 1996;19:998-1000.

18. Yano M. Epidemiology of hepatitis C virus infection in Japan. Nippon Rinsho, 1995;53 supplement:346-350 (in Japanese).

19. Tanaka J, Sasaki F, Nagakami H, Mizui M, Katayama K, Moriya H, Yoshizawa K. Estimating numbers of HBV carrier and HCV carrier in Hiroshima and Japan using data from blood donors. Nihon Koshu Eisei Zasshi 1997;44:788-796 (in Japanese).

20. Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. Hepatology, 1990;12(4 pt 1):671-675.

21. Ministry of Health and Welfare. Prevention of hepatitis B infection in health care. Recommendation from department of health policy No 37, 1987 (in Japanese).

22. Safety guideline committee for activpuncture and moxibustion. Infection prevention for an acupuncturist and a moxa therapist. In; Kobayashi H, ed. The guideline for preventing infectious disease in acupuncture and moxibustion. Meiji-Shinnkyu Collage, Kyoto, 1992:49-62 (in Japanese).