Prevalence of Inhabitants with Anti-Hepatitis C Virus Antibody in an Area Following an Acute Hepatitis C Epidemic: Age- and Area-Related Features

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The northern district of R Town, Yamagata Prefecture, Japan, experienced an epidemic of acute hepatitis C during the 6 years following 1967. A mass survey health examination for hepatitis C virus (HCV) infection was performed on the inhabitants of this district aged 6 or over (4,655 people). Of the 3,094 inhabitants (66.5%) examined, 602 (19.5%) were anti-HCV antibody (anti-HCV) positive. Of the 602 anti-HCV positive subjects, 444 (73.7%) showed an anti-HCV cut-off index of over 4.0 (high titer), 54 (9.0%) showed 3.0–4.0 (middle titer), and 104 (17.3%) showed an index of under 3 (low titer). One hundred and sixty subjects, randomly selected, were examined for HCV RNA by nested polymerase chain reaction, and positive reactions were detected in 125 subjects (78.1%). The anti-HCV positive rate differed greatly between subjects younger than 40 years (18/1,289; 1.4%) and those 40 or over (584/1,805; 32.4%). Those who had lived in this district since before the acute hepatitis C epidemic had a high rate of positive anti-HCV. Among the 47 hamlets in this farming district, the hamlet with the highest rate (71.4%) was surrounded by other hamlets where the rates decreased as the distance from the high rate hamlet increased.

The study suggested a marked regional accumulation of HCV infection in this district and an age-related difference in HCV infection rates. The cause of the accumulation was not identified, although the HCV infection in this district may have been spread during or before the acute hepatitis C epidemic and produced many HCV carriers. J Epidemiol, 1996; 6: 1-7.

hepatitis C virus, epidemiology, age, anti-HCV antibody

SUBJECTS AND METHODS

The subjects included the inhabitants, aged 6 or over, of the northern district of R Town, Yamagata Prefecture. This district is a farming area with the main crop of rice. It comprises 47 hamlets and has about 5,100 inhabitants. Of the 4,655 inhabitants aged 6 or over in this district, 3,094 (66.5%), from whom informed consent was obtained, underwent this mass survey health examination for HCV infection (Table 1).

Peripheral blood collected from each subject at the mass survey in 1991 and 1992 was centrifuged for separation of serum as soon as possible. The serum was stored at -80°C until the time of assay for anti-HCV and HCV RNA. A second-generation kit for anti-HCV: HCV EIA II Abbott (Dainabot Tokyo, Japan) was used to detect anti-HCV. When a sample showed
cut-off index (COI) of 1 or more, it was designated as anti-HCV positive. An inhibition assay using recombinant peptide antigen was performed on 200 randomly selected anti-HCV positive subjects. This recombinant peptide antigen was made from the core-region of HCV RNA, c22-3 and the NS3-NS4 region, c200 (Dainabot Tokyo, Japan)\(^3\). The grades of anti-HCV titer were divided into three groups according to the value of COI, that is, > 4 (high), 3-4 (medium) and <3 (low).

HCV RNA in serum was assayed in 160 subjects selected at random from all of the anti-HCV positive subjects. Nested polymerase chain reactions (PCR)\(^4\) were performed for HCV RNA detection. HCV genotype was assayed according to Okamoto’s method by PCR using type-specific primers\(^5\) for 10 randomly selected married couples.

All subjects were questioned as to their length of residence in the district, alcohol consumption, and surgical experiences, blood transfusion, acupuncture therapy and the use of folk remedies. The length of residence in the survey district was categorized as positive for those who had lived in the district since before the acute hepatitis epidemic and negative for those who moved there after the epidemic. A positive history of alcohol consumption was assigned to those who had consumed 360 ml or more of rice wine daily for more than 10 years. Blood sucking therapy using leeches was and still is practiced in this district, and subjects who received it were regarded as having a positive history of folk remedy use. A positive history of alcohol consumption was assigned to those who had consumed 360 ml or more of rice wine daily for more than 10 years.

RESULTS

Of the 3,094 subjects examined, 602 (19.5%) were anti-HCV positive. All of the 200 randomly selected anti-HCV positive subjects were found to be positive for inhibition assay, indicating a negligible incidence of false positives with the anti-HCV EIA II test. Therefore, nearly 20% of the inhabitants of this district aged 6 years or older had been previously infected by HCV. Of the 602 anti-HCV positive subjects, 444 (73.7%) showed an anti-HCV COI of over 4.0 (high), 54 (9.0%) showed 3.0-4.0 (medium) and 104 (17.3%) showed an index of under 3 (low). Of 160 subjects randomly selected from these 602 anti-HCV positive subjects, 125 (78.1%) were HCV RNA positive. According to the estimation by confidence intervals, the lowest limit of the 95% confidence interval was 71.7%. This suggests that 71.7% or more of the subjects are HCV RNA positive (p<0.05) and are HCV carriers. The remaining anti-HCV positive, HCV RNA negative subjects had been infected in the past, but appeared to be now free from any HCV infection. In this paper, we regarded anti-HCV positive subjects as having had the HCV infection whether or not it was persistent.

1. Prevalence of anti-HCV by sex and age

The positive anti-HCV rates were 17.9% for males and 20.8% for females, respectively (not statistically significantly different). The positive anti-HCV rate increased with age and, particularly showed a marked difference between subjects younger than 40 years (18/1, 289 ; 1.4%) and those 40 or over (584/1, 805 ; 32.4%). A similar age-related difference was also noted when males and females were analyzed separately.

To examine the relationship between the positive anti-HCV rate and the acute hepatitis epidemic period, we converted the current ages of the subjects into their ages in 1967 and separated these by two year intervals. The positive anti-HCV rate increased with age and, particularly showed a marked difference between subjects younger than 40 years (18/1, 289 ; 1.4%) and those 40 or over (584/1, 805 : 32.4%). A similar age-related difference was also noted when males and females were analyzed separately.

2. Positive anti-HCV rates by hamlet

This analysis included 1,805 subjects aged 40 or over, the age group in which the positive anti-HCV rate was higher than

| Age (years) | Male (%) | Female (%) | Total (%) |
|------------|----------|------------|-----------|
| 6-9        | 107/148 (72.3) | 117/135 (86.7) | 224/283 (79.2) |
| 10-19      | 258/325 (79.4) | 232/319 (72.7) | 490/644 (76.1) |
| 20-29      | 77/202 (38.1)  | 100/217 (46.1) | 117/419 (42.2) |
| 30-39      | 183/370 (49.5) | 215/323 (66.6) | 398/693 (57.4) |
| 40-49      | 213/335 (63.6) | 202/284 (71.1) | 415/619 (67.0) |
| 50-59      | 199/311 (64.0) | 290/346 (83.8) | 489/657 (74.4) |
| 60-69      | 258/341 (75.7) | 326/406 (80.3) | 584/747 (78.2) |
| 70-        | 123/210 (58.6) | 194/383 (50.7) | 317/593 (53.5) |
| Total      | 1418/2242 (63.2) | 1676/2413 (69.5) | 3094/4655 (66.5) |
The anti-HCV antibody positivity rate was almost zero for subjects born after 1967, increased sharply in those over 16 and increased stepwise until the age range 40-49 years.

A : The highest positivity rate was 71.4%. The hamlet showing the highest positivity rate seemed to be surrounded by hamlets in which the positivity rates decreased as distance from the central hamlet increased.

B : Hamlets with high-HCV antibody positivity rates (lower limits of the 95% confidence interval of 40% or more) were all located in the south-east part of the survey district and almost all hamlets with low positivity rates (upper limits of the 95% confidence interval of less than 40%) were located in the north.
10%. Figure 2A shows the mapping of the positive anti-HCV rates. The highest positive rate was 71.4%. We calculated the 95% confidence interval for the positive anti-HCV rate of each hamlet. Hamlets with high positive rates (lower limits of the 95% confidence interval of 40% or more) were all located in the south-east of the survey district and almost all hamlets with low positive rates (upper limits of the 95% confidence interval of less than 40%) were located in the north of the survey district (Fig. 2B).

There was only one clinic in the study area located in the hamlet where the anti-HCV positive rate was highest.

3. Anti-HCV positive rates by length of residence, alcohol consumption, drug abuse and tattooing.

These studies were performed in 1,765 subjects aged 40 or over in whom a complete interview was possible. Subjects who had lived in the survey district since before the acute hepatitis C epidemic period had a significantly (p<0.01) higher anti-HCV positive rate than those who had moved there after the epidemic, which suggests a relationship between the length of residence and the high positive anti-HCV.

The history of alcohol intake was found not to be significantly related to the anti-HCV positive rate. None of the 212 randomly selected subjects (including 94 who were anti-HCV positive) related a history of drug abuse or tattooing.

4. Anti-HCV positive rates by past history of therapy and vaccinations

The anti-HCV positive rate was significantly (p<0.01) higher in subjects with a history of blood transfusion than in those without. However, the number of positive anti-HCV subjects without a history of blood transfusion was much larger than those who did have a history. The same findings were obtained for a history of acupuncture or folk remedies (Table 3).

In Japan, vaccinations for various diseases are given, starting at infancy. In 1973, a rule of one needle per subject was...
introduced. Before then, needles had been used for more than one child, and were thus contaminated by the blood of other children. In the present study, however, positive anti-HCV rate was low for those subjects who were younger than 16 years (elementary or junior high school students) and who had received vaccinations during the acute hepatitis epidemic period, before the one needle for one subject rule was introduced (Fig.1). This makes it unlikely that the vaccination is related to the high anti-HCV positive rate in the survey district.

5. Vertical transmission of HCV: mother-sibling relationships (Table 4).

The percentage of positive anti-HCV siblings was significantly (p<0.01) higher for positive anti-HCV mothers than for negative anti-HCV mothers. However, the percentages of positive anti-HCV siblings with positive anti-HCV mothers were different by age. The percentage of positive anti-HCV siblings younger than 40 years was not as high as that in siblings aged 40 or over.

6. Sex-related transmission: husband-wife relationships (Table 5)

The percentage of positive anti-HCV partners was signifi-

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**Table 4.** Anti-HCV antibody positivity rate in children

| Anti-HCV antibody state of mothers | positive | negative |
|-----------------------------------|----------|----------|
| Total number of siblings (n=999)  | 31/217 (14.3%) | 11/782 (1.4%) |
| Siblings aged 40 years or over (n=196) | 22/83 (26.5%) | 9/113 (8.0%) |
| Siblings younger than 40 years (n=803) | 9/134 (6.7%) | 2/669 (0.3%) |

*: chi-squared test p<0.01

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**Table 5.** Analysis of married couples

(1) Anti-HCV antibody positivity rate in wives

| Anti-HCV antibody state of husbands | positive | negative |
|------------------------------------|----------|----------|
| Total number of wives (n=777)      | 101/188 (53.7%) | 82/589 (13.9%) |
| Wives aged 40 years or over (n=572) | 101/179 (56.4%) | 75/393 (19.1%) |
| Wives younger than 40 years (n=205) | 0/9 (0.0%) | 7/196 (3.6%) |

*: chi-squared test p<0.01

(2) Anti-HCV antibody positivity rate in husbands

| Anti-HCV antibody state of wives | positive | negative |
|---------------------------------|----------|----------|
| Total number of husbands (n=777) | 101/183 (55.2%) | 87/594 (14.6%) |
| Husbands aged 40 years or over (n=624) | 101/177 (57.1%) | 82/447 (18.3%) |
| Husbands younger than 40 years (n=153) | 0/6 (0.0%) | 5/147 (3.4%) |

*: chi-squared test p<0.01
Subjects of community health screening examinations increase significantly (p<0.01) higher for positive anti-HCV husbands or wives than for negative anti-HCV husbands or wives. However, when one of the married couple was younger than 40, this relationship was not observed.

Among 10 randomly selected married couples, 6 showed the same HCV RNA genotype (genotype II) and the other four showed different genotypes between wife and husband.

**DISCUSSION**

In this study, 602 subjects (19.5%) in the survey district were found to be anti-HCV positive. Kuboki et al reported that most with an anti-HCV COI of over 4 had HCV viremia. In our survey, a large majority of the anti-HCV positive subjects showed an anti-HCV COI of over 4. The detection of HCV RNA in a large majority of the positive anti-HCV subjects in this district, where there was once an epidemic of acute hepatitis C, reveals the strong possibility of a prevalence of chronic hepatitis C in this area.

In Japan, positive anti-HCV rates for blood donors and subjects of community health screening examinations increase with age, and rise sharply after the age of 40. Our study confirmed this marked increase in the positive anti-HCV rate in those aged 40 or over.

In addition to this age-related accumulation of HCV infections, a marked regional aggregation of HCV infections was also observed. Among the 47 hamlets, two had positivity rates of more than 70%. Neighboring hamlets showed the next highest positive anti-HCV rates. This shows that age and location were important factors. Another factor was the length of residence, as subjects who had lived in the district since before the acute hepatitis C epidemic had a significantly higher positive anti-HCV rate than those who had moved to the district after the epidemic. The rate was almost zero for subjects born after the epidemic period. This suggests that HCV infection in this region spread before and during the acute hepatitis C epidemic period with a few new infections occurring after the epidemic.

According to Esteban et al, HCV is transmitted by non-oral routes such as blood transfusion, drug abuse, tattooing and contaminated injection needles. Kao et al and Wejestel suggested intrafamilial infection of HCV. In our study, although the anti-HCV positivity rate differed significantly depending on the history of blood transfusion (p<0.01), acupuncture therapy (p<0.01) and the use of folk remedies (p<0.05), the absolute number of positive anti-HCV subjects with a history of these treatments was quite small. It is therefore difficult to suggest a relationship between these treatments and the increase of HCV infection. Other possible routes for HCV infection such as drug abuse, tattooing and surgery were not thought to be responsible for the high rate of HCV infection in this district.

Vaccination could be a suspected means of contamination because one single needle was used for the injection of several children before 1973 in Japan. In the present study, however, the positive anti-HCV rate was low for those who had been younger than 16 years during the acute hepatitis C epidemic, when the rule of one needle per one subject had not yet been introduced. This makes it unlikely that vaccination practice is related to the high positive anti-HCV rate in the survey district.

Needlestick injuries in clinics and hospitals are an important potential route of HCV infection to be examined. In this district, only one clinic was located in the hamlet with the highest positive anti-HCV rate. Since a marked increase of HCV infection was observed around this clinic, it could have some relationship to the route of HCV infection, although this possibility remains to be elucidated.

There was an increased rate of positive anti-HCV subjects in mother-sibling pairs if both mother-sibling were aged 40 years or over, but the increased rate was small in pairs where the sibling was younger than 40 years. Wejestel et al reported the vertical transmission of HCV. Our data did not refute the possibility of vertical transmission, but the incidence appears to be very low.

Some authors have reported a high incidence of sexual transmission of HCV, although others have reported the exact opposite. In our study, an accumulation of positive anti-HCV subjects was noted only in married couples whose age was both 40 years or over. Although the longer the couple lived together and the greater frequency of sexual contact, the more likely that both may become infected, the marked increase of the incidence around the clinic area and the factor of years spent living there suggested that inhabitants 40 years or over might have become infected from factors other than sexual contact. We examined the HCV RNA genotypes in married couples, both of whom were HCV RNA positive and aged 40 years or over. The rate of coincidence of the HCV-RNA genotype in these married couples was 60%. The HCV RNA genotype was not necessarily the same in the married couple. Therefore, it does not mean that if a married couple are both anti-HCV positive, it was due to the sexual transmission of HCV. In order to study of the sexual transmission of HCV, the precise variation of the gene sequences of HCV in married couples must be documented.

In conclusion, the present study revealed a marked regional accumulation of HCV infection in this one district and an age-related difference in infection rates. The results suggest that HCV infection in our subjects occurred during or before the acute hepatitis C epidemic. It seems unlikely that blood transfusion, drug abuse, folk remedies, vaccination or vertical transmission were responsible for HCV infection in this district.

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