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Study of the causes of higher mortality rates from chronic liver diseases in Tokushima Prefecture

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Abstract: Mortality rates from chronic liver diseases (CLD) such as liver cirrhosis and hepatocellular carcinoma have been reported to be higher in Tokushima prefecture, although its causes remain unclear. To clarify the causes of CLD in Tokushima prefecture, we evaluated the positive rates of HBs antigen and anti-HCV antibody and the mortality rates from CLD in patients with liver diseases and blood donors after dividing the entire Tokushima prefecture into 8 district boundaries of health centers. In addition, to evaluate the causes of the higher frequency of CLD and the relationship between the development of CLD and viruses, medical examinations were performed in 2 mountain villages in Tokushima prefecture where the drift of population was limited and the mortality rates from CLD differed from each other. As a result, it was found that HCV infection was the major cause of the higher mortality rates from CLD in Tokushima prefecture. Although there were marked regional differences in the mortality rates from CLD, they were mainly due to different rates of HCV infection. J. Med. Invest. 49 : 163-171, 2002

Keywords **:** *HCV, chronic liver diseases, chronic hepatitis C, regional differences, hepatocellular carcinoma*

INTRODUCTION

Following the identification of hepatitis B virus (HBV), the identification of hepatitis C virus (HCV) (1) recently facilitated the measurement of anti-HCV antibody (2) and HCV-RNA (3), resulting in the finding that most cases of non-A non-B type hepatitis were caused by HCV (4). It has been reported that hepatitis C results in the development of hepatocellular carcinoma (HCC) following the development of chronic hepatitis (CH) and liver cirrhosis (LC).

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Mortality rates from CLD have always ranked higher in Tokushima prefecture than in other urban and rural prefectures in Japan (5). For example, the mortality rates from CLD of Tokushima and Japan were 22.8 and 13.7 per 100,000 in 1990. Then Tokushima ranked first among the prefectures in Japan. The tendency was almost the same from 1955 to 1995. To evaluate the etiology of CLD in Tokushima prefecture, Nose *et al*. investigated HBs antigen in autopsied cases of CH, LC, and HCC, and reported that the positive rate of HBs antigen was higher in the respective liver diseases (6). Although some studies have suggested a relationship between CLD and HBV, there have been no studies evaluating the relationship between CLD and HCV in Tokushima prefecture.

Since the relationship between CLD and HCV has

recently been clarified in geographical areas showing higher mortality rates from CLD, localization of the increased frequency of CLD has been reported to date (7). Although these findings suggest that HCV infection may be a cause of higher mortality rates from CLD in Tokushima prefecture, their major cause remains unclear.

Therefore, we evaluated the positive rates of HBs antigen and anti-HCV antibody and mortality rates from CLD in patients with liver diseases and blood donors after dividing Tokushima prefecture into 8 district boundaries of health centers. In addition, to evaluate the cause of the increased frequency of CLD and the relationship between the development of CLD and viral infection rates, medical examinations were performed in 2 mountain villages where mortality rates from CLD differed from each other and the drift of population was limited.

SUBJECTS AND METHODS

Subjects

1) Liver disease group :

The liver disease group consisted of 815 patients in whom HBs antigen and second-generation anti-HCV antibody were measured after establishing definitive diagnoses of AH, CH, LC, and HCC at Tokushima University Hospital and other related hospitals in Tokushima Prefecture between April 1990 and March 1997.

2) Permanent resident group :

The permanent resident group consisted of 351 of 815 patients with liver diseases who had been settled in one confirmed district in Tokushima Prefecture for more than 20 years.

3) Local resident group :

The local resident group consisted of 241 subjects (106 residents of A village and 135 residents of B village) who had lived in the respective villages for 20 years (1972-1992). In these 2 villages, the drift of population was limited and mortality rates from CLD per 100,000 population differed (22.7 in A village vs. 99.4 in B village) (8).

Methods

1) Diagnosis of liver diseases :

Diagnoses of the respective liver diseases (AH, CH, LC, and HCC) were established based on the results of hematological examinations, abdominal ultrasonography, and CT, in addition to the results of liver biopsy and angiography performed when necessary. Patients who were positive for HBs antigen were diagnosed as having hepatitis B, while those who were positive for anti-HCV antibody were diagnosed as having hepatitis C. In addition, those who had continuously been drinking more than 600 ml of alcohol a day for more than 5 years were diagnosed as having alcoholic liver injury based on the results of interview (9).

2) Evaluation in the permanent resident group :

After dividing the entire Tokushima Prefecture into 8 district boundaries of health centers, correlation among mortality rates from CLD (8) and positive rates of HBs antigen and anti-HCV antibody (10) were evaluated in patients with liver diseases and blood donors.

3) Evaluation in the local resident group

To detect liver diseases, levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-GTP, HBs antigen, and anti-HCV antibody were measured in the local resident group (A and B villages). In addition, abdominal ultrasonography was performed when necessary.

Local residents who showed AST or ALT levels higher than the upper limit of the normal value were classified as an abnormal liver function group, whereas those who showed normal AST and ALT levels were classified as a normal liver function group. In each group, positive rates of HBs antigen and anti-HCV antibody were evaluated by age.

4) Evaluation of mortality rates from CLD

Mortality rates from CLD were obtained based on cause of death statistics (5) and annual health report statistics in Tokushima Prefecture (8).

5) Statistical analysis

Mean values were statistically analyzed using Student's t-test, and p-values less than 0.05 were considered significant. In addition, correlation analysis was performed using Pearson's correlation coefficients, and p-values less than 0.05 were considered significant.

RESULTS

1. Evaluation in the liver disease group

Table 1 shows the clinical data of the liver disease group and the results of virological study. Excluding patients with AH, the positive rates of anti-HCV antibody in patients with CH, LC, and HCC were 76.4%, 57.3%, and 70.5%, respectively. HBV infection was involved in less than 20% of CLD patients since the positive rates of HBs antigen in patients with CH, LC, and HCC were 12.0%, 18.0%, and 18.9%, respectively. Frequencies of double infection with HBV and HCV in patients with CH, LC, and HCC were 1.0%, 1.7%, and 25.3%, respectively. Frequencies of infection with non-B non-C type liver disease in patients with CH, LC, and HCC were 5.2%, 6.7%, and 4.2%, respectively. Moreover, frequencies of alcoholic liver injury in patients with CH, LC, and HCC were 2.5%, 12.4%, and 0%, respectively. Therefore, positive rates of anti-HCV antibody were highest in patients with CH, LC, or HCC.

2. Evaluation in the permanent resident group

Table 2 shows the clinical data of the permanent resident group. Positive rates of HBs antigen and anti-HCV antibody in permanent residents with CH, LC, and HCC were similar to those in the liver disease group. That is, positive rates of anti-HCV antibody in permanent residents with CH, LC, and HCC were 69.1%, 54.5%, and 70.7%, respectively. Positive rates of HBs antigen in permanent residents with CH, LC, and HCC were 13.0%, 14.3%, and 17.1%, respectively. Frequencies of double infection with HBV and HCV in permanent residents with CH, LC, and HCC were 1.3%, 1.3%, and 7.3%, respectively. In addition, frequencies of infection with non-B non-C type liver disease in permanent residents

Table 1. Clinical data of the liver disease group

	No. of the patients	HBs-Ag(+)	HCV-Ab(+)	HBs-Ag(+) + HCV-Ab(+)	nonB, nonC	Alcohol	Other
Acute hepatitis	25	3 (12.0)	2 (8.0)	0 (0.0)	3 (12.0)	0 (0.0)	17 (68.0)
Chronic hepatitis	517	62 (12.0)	395 (76.4)	5 (1.0)	27 (5.2)	13 (2.5)	15 (2.9)
Liver cirrhosis	178	32 (18.0)	102 (57.3)	3 (1.7)	12 (6.7)	22 (12.4)	7 (3.9)
Hepato cellular carcinoma	95	18 (18.9)	67 (70.5)	6 (6.3)	4 (4.2)	0 (0.0)	0 (0.0)
Total (%)	815	115 (14.1)	566 (69.4)	14 (1.7)	46 (5.6)	35 (4.3)	39 (4.8)

Table 2. Clinical data of the permanent resident group

	No. of the patients	HBs-Ag(+)	HCV-Ab(+)	HBs-Ag(+) + HCV-Ab(+)	nonB, nonC	Alcohol	Other
Acute hepatitis	10	1 (10.0)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	8 (80.0)
Chronic hepatitis	223	29 (13.0)	154 (69.1)	3 (1.3)	19 (8.5)	8 (3.6)	10 (4.5)
Liver cirrhosis	77	11 (14.3)	42 (54.5)	1 (1.3)	5 (6.5)	13 (16.9)	5 (6.5)
Hepato cellular carcinoma	41	7 (17.1)	29 (70.7)	3 (7.3)	2 (4.9)	0 (0.0)	0 (0.0)
Total (%)	351	48 (13.7)	225 (64.1)	7 (2.0)	27 (7.7)	21 (6.0)	23 (6.6)

Table 3. Positive rates of HBs antigen and anti-HCV antibody in liver disease patients and blood donors classified by district boundaries of health centers and mortality rates from CLD per 100,000 population in Tokushima prefecture.
① Ikeda, ② Anabuki, ③ Kamojima, ④ Naruto, ⑤ Tokushima, ⑥ Komatsushima, ⑦ Anan, ⑧ Hiwasa

Area	Patients with liver disease		Blood donor		Rate of death from liver disease (per 100,000)
	HBs-Ag(+) (%)	HCV-Ab(+) (%)	HBs-Ag(+) (%)	HCV-Ab(+) (%)	
①	16.7	41.7	0.58	0.97	20.3
②	7.8	72.5	0.60	1.17	29.4
③	12.7	70.9	0.35	1.65	22.2
④	8.5	72.3	0.27	1.11	25.4
⑤	12.1	68.1	0.35	1.11	20.6
⑥	14.1	72.9	0.42	1.11	22.4
⑦	21.3	72.3	0.64	1.42	18.7
⑧	16.6	41.6	0.52	1.97	16.6
Average	13.7±4.5	64.0±13.9	0.46±0.14	1.31±0.34	22.0±4.0

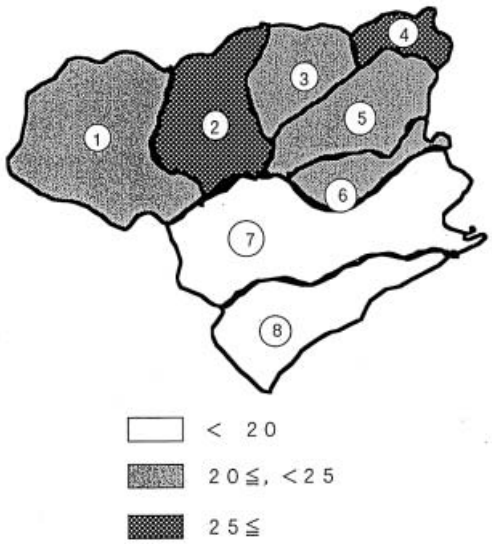

Figure 1. Mortality rates from chronic liver diseases in Tokushima prefecture classified by district boundaries of health centers (per 100,000 population, ①-⑧ correspond to Table 3).

with CH, LC, and HCC were 8.5%, 6.5%, and 4.9%, respectively. Moreover, frequencies of alcoholic liver injury in permanent residents with CH, LC, and HCC were 3.6%, 16.9%, and 0%, respectively. Therefore, the positive rates of anti-HCV antibody were highest in permanent residents with CH, LC, or HCC.

Table 3 shows the positive rates of HBs antigen and anti-HCV antibody in permanent residents and blood donors classified by district boundaries of health centers and mortality rates from CLD per 100,000 population in Tokushima Prefecture. As shown in Figure 1, mortality rates from CLD tended to be higher in the northern part and lower in the southern part of Tokushima Prefecture. Figure 2 shows the positive rates of HBs antigen and anti-HCV antibody in blood donors, while Figure 3 shows the positive rates of HBs antigen and anti-HCV antibody

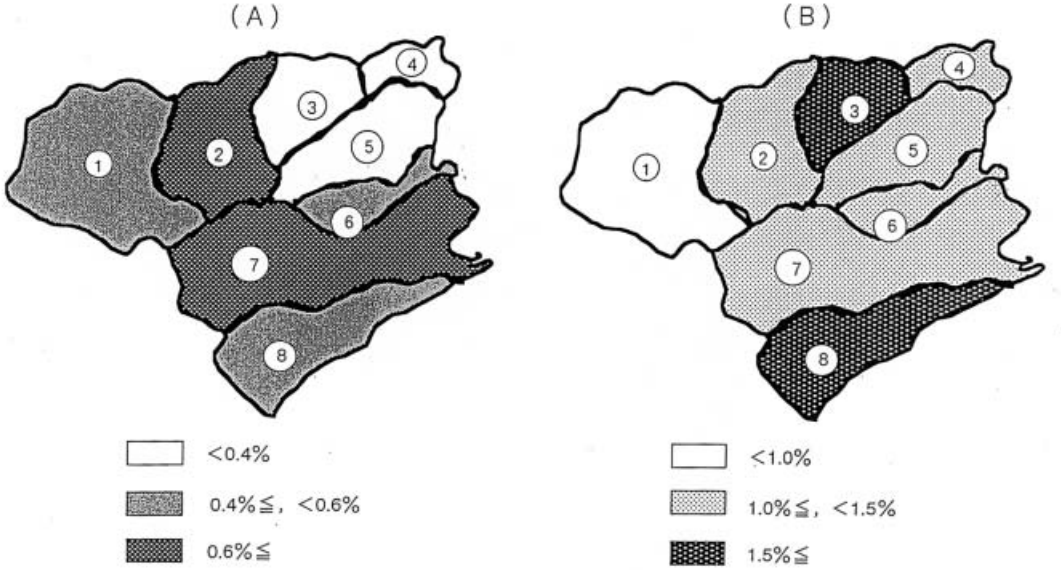

Figure 2. Percentages of blood donors who were positive for HBs antigen (A) or anti-HCV antibody (B) classified by district boundaries of health centers in Tokushima Prefecture (①-⑧ correspond to Table 3).

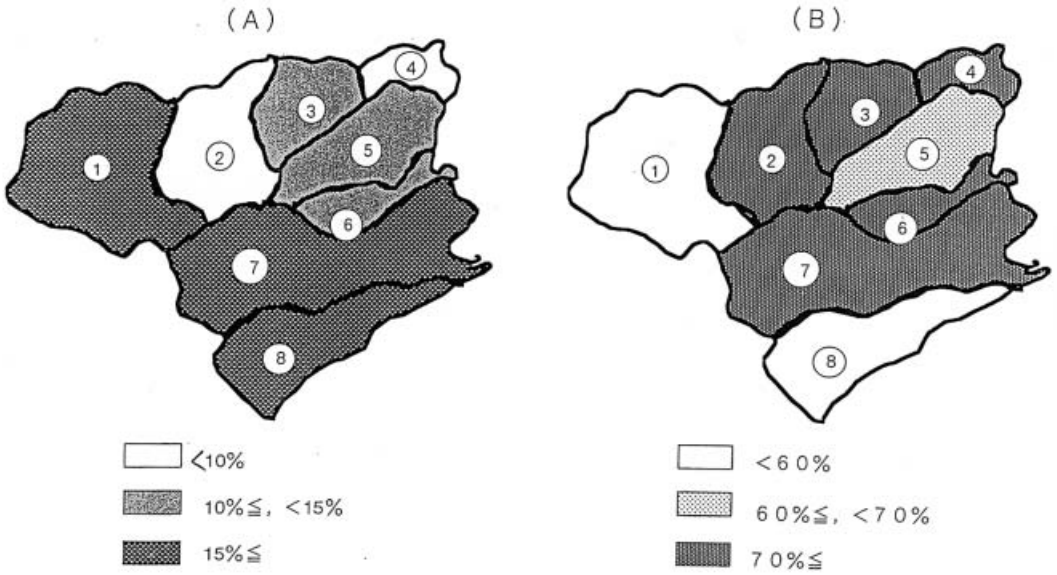

Figure 3. Percentages of permanent residents who were positive for HBs antigen (A) or anti-HCV antibody (B) classified by district boundaries of health centers in Tokushima prefecture (①-⑧ correspond to Table 3).

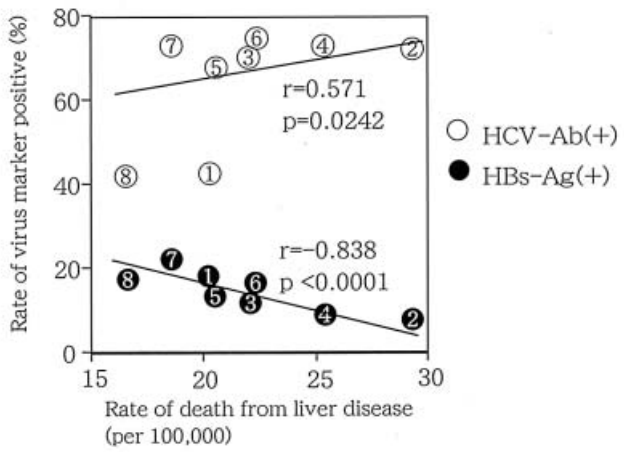

Figure 4. Correlation among positive rates of HBs antigen and anti-HCV antibody in liver disease patients classified by district boundaries of health centers and mortality rates from CLD per 100,000 population in Tokushima prefecture.

in permanent residents. The percentage of HBs antigen-positive blood donors was higher than 0.6% in districts ② and ⑦, while that of anti-HCV antibody-positive blood donors was higher than 1.5% in districts ③ and ⑧. When mortality rates from CLD were compared to the positive rates of HBs antigen and anti-HCV antibody, the percentages of CLD patients who were positive for anti-HCV antibody tended to be higher in districts ② and ④ where mortality rates from CLD were higher (72.5% and 72.3%, respectively). However, the positive rate of HBs antigen did not show such a tendency. The percentages of blood donors who were positive for HBs antigen and anti-HCV antibody were 0.46% and 1.38%, respectively. However, the positive rates of HBs antigen and anti-HCV antibody did not significantly correlate with the mortality rates from CLD in blood donors classified by the district boundaries of health centers. In patients with CLD, the positive rate of anti-HCV antibody was higher in the northern part of Tokushima Prefecture, as were the mortality rates from CLD. Figure 4 shows the correlation among positive rates of HBs antigen and anti-HCV antibody in liver disease patients classified by district boundaries of health centers and mortality rates from CLD per 100,000 population in Tokushima Prefecture. The percentage of HBs antigen-positive patients was lower in geographical areas where mortality rates from CLD were higher, although the percentage of anti-HCV antibody-positive patients tended to be higher (correlation coefficients were -0.838 and +0.571, respectively). These findings suggest that hepatitis C, not hepatitis B, may greatly contribute to higher mortality rates from CLD.

3. Evaluation in the local resident group

Table 4 shows the summary of local residents of 2 mountain villages where the drift of population was relatively limited. The percentages of local

Table 4. Summary of local residents in villages A and B

	village A	village B
date	1993.8.20	1994.9.3-4
number	106	131
age (average)	28～82(59.6)	27～78(59.4)
sex (male/female)	66/40	66/65
number of abnormal liver function test (%)	47(44.3)	55(42.0)

Table 5. Numbers of local residents of villages A and B with and without normal liver function test who were positive for HBs antigen or anti-HCV antibody.

	abnormal liver function group					normal liver function group				
	HBs-Ag(+) HCV-Ab(−)	HBs-Ag(+) HCV-Ab(+)	HBs-Ag(−) HCV-Ab(+)	HBs-Ag(−) HCV-Ab(−)	Total (%)	HBs-Ag(+) HCV-Ab(−)	HBs-Ag(+) HCV-Ab(+)	HBs-Ag(−) HCV-Ab(+)	HBs-Ag(−) HCV-Ab(−)	Total (%)
village A (n=106)	1 (2.1)	1 (2.1)	9* (19.2)	36 (76.6)	47 (100.0)	6 (10.2)	0 (0.0)	6* (10.2)	47 (79.7)	59 (100.0)
village B (n=131)	0 (0.0)	0 (0.0)	46* (83.6)	9 (16.4)	55 (100.0)	0 (0.0)	0 (0.0)	26* (34.2)	50 (65.8)	76 (100.0)
Total (n=237)	2 (2.0)	0 (0.0)	55 (53.9)	45 (44.1)	102 (100.0)	6 (4.4)	0 (0.0)	32 (23.7)	97 (71.9)	135 (100.0)

*p<0.05

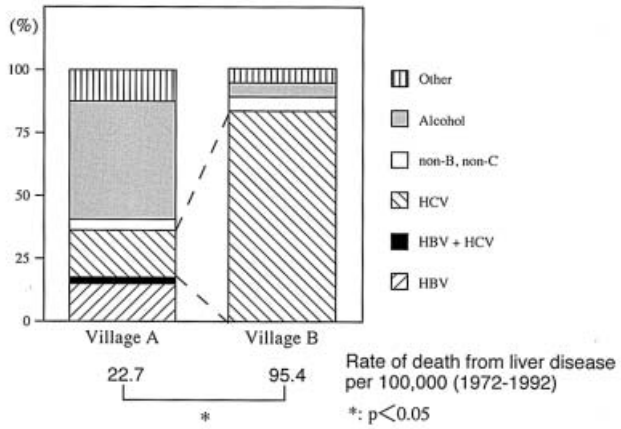

Figure 5. Clinical data of local residents without normal liver function test in villages A and B classified by the respective causes.

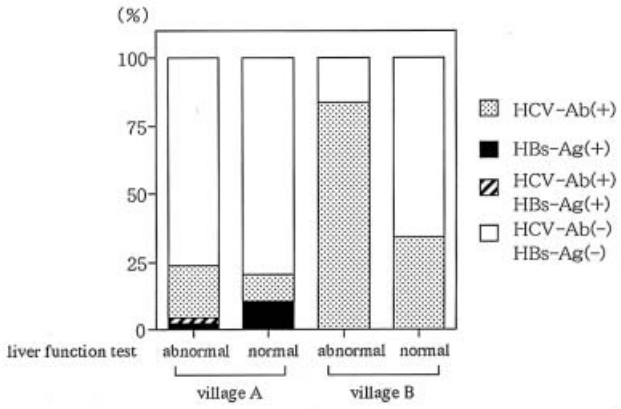

Figure 6. Positive rates of HBs antigen and anti-HCV antibody in local residents with and without normal liver function test in villages A and B.

residents with abnormal liver function test were 44.3% in village A and 42.0% in village B. Table 5 shows numbers of local residents of villages A and B with and without normal liver function test who were positive for HBs antigen or anti-HCV antibody. Figure 5 shows the clinical data of local residents without normal liver function test in villages A and B classified by the respective causes. Although local residents with alcoholic liver injury accounted for approximately 50% of those with liver failure in village A, more than 80% of those with liver failure were positive for anti-HCV antibody in village B.

Figure 6 shows the positive rates of HBs antigen and anti-HCV antibody in local residents with and without normal liver function test in villages A and B. The positive rate of anti-HCV antibody tended to be higher in village B than in village A, regardless of the presence or absence of liver failure. Figures 7 and 8 show the numbers of patients with and without normal liver function test who were positive for HBs antigen or anti-HCV antibody classified by age in villages A and B. In village A, none of the residents below 50 years old were positive for HBs antigen or anti-HCV antibody, although some residents of 50 years old or over were positive for HBs antigen or anti-HCV antibody. In village B, however, none of the residents were positive for HBs antigen, and residents who were positive for anti-HCV antibody were widely distributed in all age groups. Although mortality rates from CLD differed among the respective age groups, mean mortality rates from CLD over the past 20 years were 22.7 in village A and 95.4 in village B (8). Thus, the mean mortality rate from CLD in village B was approximately 4 times higher than that of village A. The mean mortality rate from CLD was markedly higher in village B than in the entire Tokushima prefecture (22.0), although it was similar between village A and the entire Tokushima prefecture. In addition, the higher incidence of hepatitis C was positively correlated with mortality rates from CLD (r=0.571, p=0.1436).

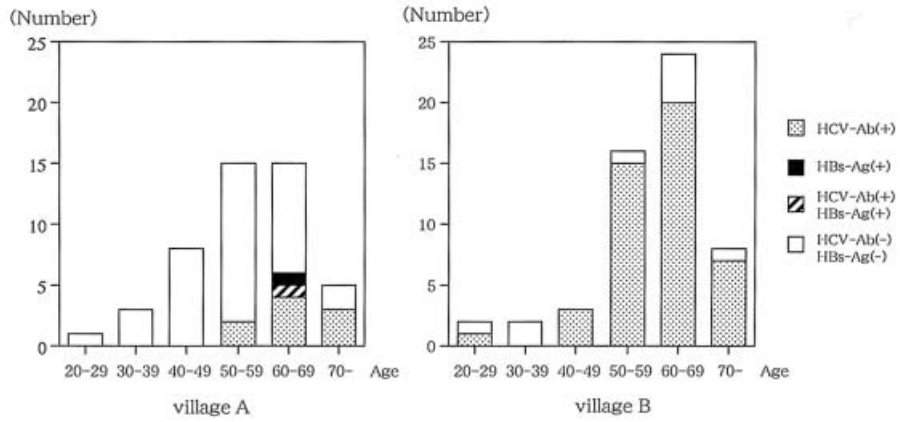

Figure 7. Numbers of patients without normal liver function test who were positive for HBs antigen or anti-HCV antibody classified by age in villages A and B.

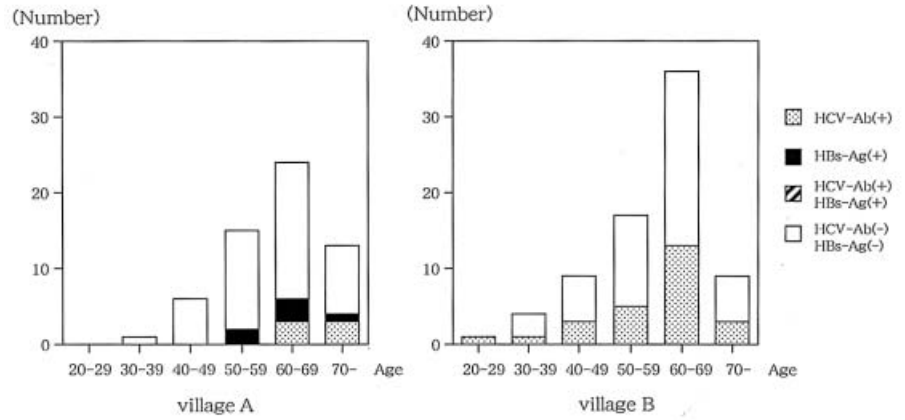

Figure 8. Numbers of patients with normal liver function test who were positive for HBs antigen or anti-HCV antibody classified by age in villages A and B.

DISCUSSION

Recently, mortality rates from CLD such as LC and HCC have gradually been increasing, and most cases of death from CLD are thought to be caused by HCV. According to cause of death statistics (5), mortality rates from CLD always ranked higher (1st-5th places) in Tokushima prefecture than in other prefectures in Japan between 1955 and 1995 (8), demonstrating that the incidence of CLD is highest in Tokushima Prefecture. However, few studies have evaluated the details of higher mortality rates from CLD in Tokushima Prefecture. Therefore, we performed an epidemiological study in Tokushima Prefecture after dividing the entire Tokushima Prefecture into 8 district boundaries of health centers, and the positive rates of HBs antigen and anti-HCV antibody and mortality rates from CLD were evaluated in patients with liver diseases and blood donors. In addition, to evaluate the cause of the increased frequency of CLD and the relationship between the development of CLD and viral infection rates, medical examinations were performed in 2 mountain villages where the drift of population was limited. The positive rates of HBs antigen in autopsied cases of CH, LC, and HCC reported by Nose *et al*. were 16.3%, 54.0%, and 60.0%, respectively. In this study, however, the positive rates of blood HBs antigen in patients with CH, LC, and HCC were 12.0%, 18.0%, and 18.9%, respectively. In addition, the positive rates of blood anti-HCV antibody in patients with CH, LC, and HCC were 76.4%, 57.3%, and 70.5%, respectively. Thus, the positive rates of blood anti-HCV antibody were higher than those of HBs antigen. Similar positive rates of anti-HCV antibody were obtained in permanent residents with liver diseases. Although the positive rates of HBs antigen in this study tended to increase with the development of CH to HCC via LC, they were lower than those reported by Nose *et al*. Since the positive rates of HBS antigen in the blood may strongly correlate with those in the tissue, increased positivity for HBs antigen may not be due to sample differences. Therefore, this finding may suggest that the incidence of hepatitis C markedly increased during the 1990s compared to that during the 1970s. Indeed, Nishioka

et al. (4) evaluated yearly changes in the number of HCC patients, and reported that the number of patients with hepatitis C tended to increase markedly, although the number of patients with hepatitis B did not significantly vary.

National statistics revealed that both the mortality rates from CLD (5) and the positive rates of anti-HCV antibody in blood donors tended to be higher in the western part and lower in the eastern part of Japan, suggesting regional differences in the mortality rates from CLD and positive rates of anti-HCV antibody. Therefore, to further evaluate the regional differences in Tokushima Prefecture, we evaluated permanent residents classified by district boundaries of health centers. In addition, the incidence of CLD was investigated in 2 mountain villages where the drift of population was limited and mortality rates from CLD differed each other to compare the results with the statistics of CLD in Tokushima Prefecture. Although the positive rate of anti-HCV antibody tended to be higher in the 2 mountain villages where mortality rates from CLD were high, the percentage of HBs antigen-positive residents was lower in these 2 districts than in other districts in Tokushima prefecture. In addition, the positive rates of both HBs antigen and anti-HCV antibody did not correlate with mortality rates from CLD in blood donors. The evaluation of local residents revealed that the major causes of abnormal liver function were alcohol in village A and HCV infection in village B. Mortality rates from CLD were also higher in village B where the positive rate of anti-HCV antibody was high. Moreover, it was found that the positive rate of anti-HCV antibody was high in local residents with and without normal liver function in village B where mortality rates from CLD were high, suggesting that the positive rate of anti-HCV antibody tend to be higher in elderly subjects. However, both hepatitis B and C were only slightly involved in residents with and without normal liver function in village A where mortality rates from CLD were low, demonstrating that alcohol was the major cause of the abnormal liver function in this village. From these findings, it was considered that HCV was the main cause of liver disease progression compared with alcohol only in these villages.

These findings suggest that HCV infection may play an important role in the higher mortality rates from CLD in Tokushima prefecture. Previously, HBV has been thought to play an important role in the higher incidence of CLD in Tokushima prefecture. However, it has recently been demonstrated that most cases of CLD in Tokushima prefecture were caused by HCV, and there were marked regional differences in the incidence of CLD. Although there were no marked regional differences in the positive rate of HBs antigen either in the whole of Japan or in Tokushima prefecture, the positive rate of anti-HCV antibody tended to be higher in the western part and lower in the eastern part of Japan. In Tokushima prefecture, the positive rate of anti-HCV antibody tended to be higher in the northern part and lower in the southern part. Furthermore, it was found that the positive rate of anti-HCV antibody was marked higher in some local areas in Tokushima prefecture. These findings suggest that there are regional differences in HCV infection pathways, although they were not evaluated in this study.

After the introduction of anti-HCV antibody screening for blood donors, the incidence of post-transfusion hepatitis has markedly decreased (11). In particular, the development of post-transfusion hepatitis has rarely been reported since the introduction of second-generation anti-HCV antibody (12). Recently, the positive rate of anti-HCV antibody in blood donors in Tokushima prefecture (1.2%) has been similar to that in the whole of Japan (1.0%), and the incidence of chronic hepatitis C may further decrease in the future. Therefore, evaluating the incidence of hepatitis C by medical examinations in all the districts of Tokushima prefecture to prevent HCV infection may further decrease mortality rates from CLD.

CONCLUSIONS

1. Epidemiological and clinical investigations of liver diseases revealed that hepatitis C was the major cause of higher mortality rates from chronic liver diseases in Tokushima prefecture.
2. It was found that hepatitis C plays an important role in the regional differences in mortality rates from chronic liver diseases in Tokushima Prefecture.
3. In the future, the decreased frequency of post-transfusion hepatitis may decrease mortality rates from chronic liver diseases.

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