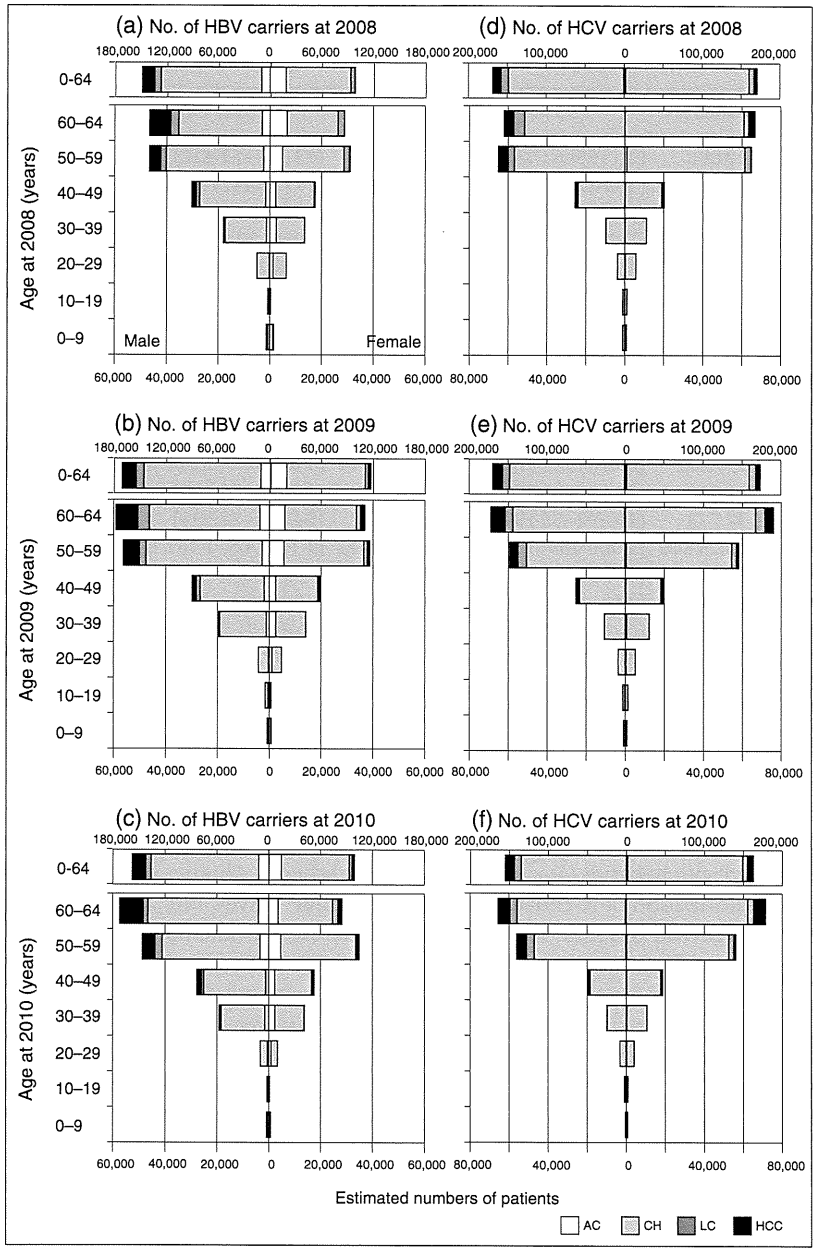

Figure 6 Number of persons who carried hepatitis B virus (HBV) or hepatitis C virus (HCV). Sex- and age-specific distributions of HBV or HCV are shown. (a) Number of HBV carriers at 2008. (b) Number of HBV carriers at 2009. (c) Number of HBV carriers at 2010. (d) Number of HCV carriers at 2008. (e) Number of HCV carriers at 2009. (f) Number of HCV carriers at 2010. (a) AC, asymptomatic carriers; CH, chronic hepatitis; HCC, hepatocellular carcinoma; LC, liver cirrhosis.

DISCUSSION

BECAUSE OF THE paucity of subjective symptoms, persons persistently infected with HBV and HCV are either undiagnosed or diagnosed. Tanaka *et al.* previously estimated that 903 000 asymptomatic carriers were persistently infected with HBV and 808 000 asymptomatic carriers were persistently infected with HCV. They all were

Table 4 Estimated number of patients under 65 years

		2008		2009		2010	
		Patients	(95% CI)	Patients	(95% CI)	Patients	(95% CI)
HBV-related chronic liver disease†	AC	29 086	(10 186–61 435)	29 109	(13 354–53 427)	25 950	(11 579–48 925)
	CH	192 641	(139 823–250 594)	226 601	(177 234–277 366)	203 278	(158 453–249 952)
	LC	11 933	(1420–38 051)	12 485	(3501–31 265)	9498	(1520–27 593)
	HCC	14 569	(5879–42 267)	19 155	(9090–39 895)	18 340	(8599–37 368)
	Total	248 229	(194 727–301 864)	287 350	(240 013–334 686)	257 065	(213 777–300 402)
HCV-related chronic liver disease†	AC	2006	(0–27 012)	2635	(127–18 621)	2104	(0–17 023)
	CH	306 877	(239 614–378 855)	305 028	(247 709–363 663)	282 438	(229 413–337 131)
	LC	16 376	(3 637–44 398)	17 511	(6 734–37 971)	14 830	(5 003–34 774)
	HCC	14 755	(3 958–41 904)	18 624	(7 818–39 892)	19 528	(8 639–39 470)
	Total	340 014	(276 789–403 239)	343 798	(291 539–396 058)	318 900	(270 065–367 735)
HBV-related or HCV-related or other chronic liver disease†	AC	31 092	(11 542–50 914)	31 653	(15 244–48 158)	27 966	(12 946–43 103)
	CH	997 442	(890 019–1 104 865)	1 117 944	(1 024 775–1 211 113)	1 046 460	(958 453–1 134 468)
	LC	57 538	(32 137–83 372)	60 815	(39 327–82 600)	52 659	(33 138–72 413)
	HCC	37 773	(19 692–59 197)	51 610	(34 066–71 334)	48 762	(31 764–67 355)
	Total	1 123 846	(1 009 469–1 238 222)	1 262 022	(1 162 905–1 361 138)	1 175 847	(1 082 365–1 269 330)
Acute hepatitis A		2379	(0–11 875)	1800	(0–9379)	1830	(75–9352)
Acute hepatitis B		3837	(247–15 275)	1961	(178–10 471)	2726	(16–9374)
Acute hepatitis C		385	(0–12 831)	93	(0–8917)	720	(0–8108)

†Including patients co-infected with HBV and HCV.
AC, asymptomatic carriers; CH, chronic hepatitis; CI, confidence interval; HCC, hepatocellular carcinoma; LC, liver cirrhosis.

identified as first-time blood donors during 2005 in Japan, and were unaware of their infections.

In this study, we estimated the number of patients who have already been diagnosed with hepatitis virus infections by analysis based on the information of re-coded medical claims including several diagnosed diseases. The medical claim is a claim from health-care facilities sent to insurance companies or the government upon payment for medical treatment which the patient has actually received. On a medical claim, for each insured patient, there is information about the patient including name, sex and date of birth, and health insurance details, together with monthly basic information of the health-care facility in which the original medical claim was made, including the name of the institution, department, disease name, number of prescriptions every month, and treatment received such as injections, medication, surgery, medical examination, imaging diagnosis and rehabilitation.

The JMDC is commissioned by the Health Insurance Society, an insurance society in Japan including approximately 25% of the entire Japan population. The JMDC has a database of health insurance medical claims, and the anonymous data are available to third parties which have a contract with the Health Insurance Society.[4] An advantage of the analysis using medical claims is that all medical information of patients belonging to the Health Insurance Society, including even their family members, is available. Because Japan has universal health insurance, practically all Japanese have joined the public health insurance scheme. Another advantage is that this study was not a sample survey but a complete enumeration of data. Therefore, the time-dependent changes in classified disease were able to be identified and dealt with accordingly.

However, generally, the results of laboratory tests and treatments were described in the medical claim, while various expressions of the name of disease might have been problematic. Also, because of the limitation of classification of diseases and the standards of insurance coverage, various expressions of the disease might have been used to indicate the same pathological condition. Thus, the number of patients could not be estimated by using the medical claim database itself.

However, in this study, based on the information of drugs and treatments retrieved from each patient's medical claim, and the natural course of their disease, a decision on the disease reclassification was made on discussions with specialists including two hepatologists. On the basis of these data, the numbers of patients with liver disease were subsequently estimated. As a result, the estimated numbers of patients under 65 years with persistent hepatitis virus infections including those with unidentified viruses were 1 123 846–1 262 022. These figures were twice that

of the estimated numbers of patients infected with hepatitis viruses. We could not recognize the transition of patients because of our short study period, but we could still distinguish between AC and CH during a follow-up period of several months to 1 year.

So far, there have been several reports analyzing medical payment records (medical claims).[5–11] Many studies of medical claims estimated the health-care costs.[12–18] There were a few reports using the medical claim database with small sample sizes for estimating the numbers of patients with other diseases such as measles,[6] acute hepatitis morbidity,[7] diabetes[8] and arthritis.[9]

On the other hand, "patient surveys", which are held every 3 years, are only to estimate the number of patients in Japan. In patient surveys, the number of patients is estimated based on the number of inpatient and outpatient visits to clinics or hospitals in 1 day out of 3 days in October, which are randomly selected from hospitals all over Japan. In case of estimation of hepatic disease, results from patient surveys may be an underestimation caused by two factors. First, a primary disease is only counted for one patient. Second, a patient whose interval between visits to clinics and hospitals is 31 days or over is excluded from the survey. The number of patients in the 2008 Patient Survey is shown in Table 5. The numbers of patients with HBV-related chronic liver disease and HCV-related chronic liver disease in this study were 192 641 and 306 877, respectively. The numbers of patients with HBV-related chronic liver disease and HCV-related chronic liver disease in the 2008 Patient Survey was 52 000 and 260 000, respectively. These results indicate that the number of patients in our study with AC or CH is estimated to be higher even under 65 years than in the 2008 Patient Survey in all ages.

Moreover, in this study we also estimated the number of patients with acute hepatitis. According to the Infectious Disease Surveillance Center (IDSC) in Japan during each year from 2008 to 2010, numbers of patients with acute hepatitis A, acute hepatitis B and acute hepatitis C were reported by medical doctors as 115–347, 174–178 and 39–52, respectively. It is regrettably said that the reported number from mandatory surveillance system could only account for 10% of cases of acute hepatitis. Estimates in this study counted 10–30 times of the reported number from the IDSC. On the other hand, along with the incidence rate of HCV infection among blood donors during the 10 years between 1994 and 2004 in Hiroshima, Japan, calculated as 1.86/100 000 person-years,[19] approximately 3335 (95% confidence interval, 483–20 866) of persons newly infected with HCV without symptoms could be estimated in a year. Thus, to prevent the outbreak of infectious diseases, it seems necessary to be aware of the fact that physicians are obligated to notify the IDSC whenever they diagnose AH according to the Law of Infectious Disease in Japan.

There are limitations in this study. First, we only estimated the number of patients aged under 65 years. However, because the onset of HCC and LC occur more frequently in persons aged over 65 years, considering the number of patients with HCC and LC of all ages, the estimated numbers of HCC and LC in our study may appear to be small. On the other hand, the number of patients with LC and HCC from patient survey may be supplemented in the estimation over 65 years, because patients with LC and HCC are consulted mostly. Second, there are some issues on the validity of the disease on the medical claims; that is, sometimes medical claims are only for clinical laboratory examination fee remuneration from insurance, and there are discrepancies between diagnosed disease on medical claims and ICD classification.

Table 5 Number of patients in the 2008 Patient Survey and infection surveillance in the Infectious Disease Surveillance Center (IDSC)

Survey	Disease (ICD)	No. of patients		
		2008	2009	2010
2008 Patient Survey	Malignant neoplasm of liver and intrahepatic bile ducts (C22)	66 000	–	–
	Liver cirrhosis (K74.3–K74.6)	59 000	–	–
	Chronic hepatitis (K73)	74 000	–	–
	Disease of liver† (K70-K77)	247 000	–	–
	Hepatitis B virus (B16–B17.0 B18.0–B18.1)	52 000	–	–
	Hepatitis C virus (B17.1 B18.2)	260 000	–	–
Infection surveillance in IDSC	Acute hepatitis A	169	115	347
	Acute hepatitis B	178	178	174
	Acute hepatitis C	52	40	39

†"Disease of liver" contains "liver cirrhosis" and "chronic hepatitis".

However, the medical claims from insurance policies are regarded as a powerful tool with sufficient reliability for estimating the number of patients with any specific disease, in cases where the tentative diagnosis from only clinical laboratory examination were excluded completely or the discrepancy between diagnosed disease on medical claim and ICD classification were adjusted properly. We determined the specific disease by constructing a theoretical flowchart for calculation and evaluating time series medical claims sorted by case ID for 3 years. This estimation method and protocol minimized the disadvantage of medical claim analysis. Thus, it should be noted that the estimates calculated in this study are possible to estimate the number of patients aged 64 years or younger precisely. Furthermore, because the number of subjects aged 60–64 years is small, the 95% confidence interval of estimated number of patients in this population ranged widely. Finally, there is a limitation of healthy worker bias. Because the estimation in this study was based on the information of re-coded medical claim of employees and their families in only 20 health insurance associations, the estimated number may be underestimated.

According to our analysis, the estimated numbers of patients aged under 65 years who have already been diagnosed with liver disease caused by HBV or HCV excluding duplicated cases were 563 688–617 421, and those caused by HBV, HCV or other chronic liver disease were 1 123 846–1 262 022. On the other hand, the estimated number of patients with undiagnosed HBV or HCV infection among first-time blood donors aged 15–69 years in 2005 was 905 397. In addition, there may be many carriers who have never been consulted or who have not been continuously treated in spite of awareness of their infection. Taken together, it is supposed that the total number of patients with liver disease is much higher than the estimated number in patients under 65 years of age. In patient survey in Japan, the number of patients is estimated by the main disease in one patient even though the patient has been diagnosed with several diseases. Based on the database with hepatitis-related diseases after evaluating several diagnosed diseases from medical claims, the estimation method and protocol may minimize the disadvantage of medical claim analysis, and are useful for patients especially with AC and CH which had been underestimated by patient survey.

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Hepatology Research

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Original Article

Hepatitis B virus infection in hemodialysis patients in Japan: Prevalence, incidence and occult hepatitis B virus infection

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Aim: A survey of hepatitis B virus (HBV) infection in hemodialysis (HD) patients was conducted to determine the burden and risk of infection and to suggest preventive measures against HBV infection among HD patients at nine hospitals in Hiroshima, Japan, from 1999 to 2003.

Methods: HBV markers were investigated for 1860 HD patients. The prevalence, incidence of HBV and prevalence of occult HBV were calculated.

Results: The prevalence of hepatitis B surface antigen (HBsAg) was 2.6%, the positive rate of anti-hepatitis B core (HBc) was 20.6% and that of anti-hepatitis B surface (HBs) was 11.7%. Among 1372 patients who started HD after the approval of erythropoietin in Japan in 1991, the prevalence of HBsAg was 2.1%. The incidence rate of HBsAg positivity was 0/1000 person-years and the incidence of anti-HBc was 0.3/1000 person-years. Among 1812 HBsAg negative patients HBV DNA was detected in two: one case was negative for anti-HBc and anti-HBs, and the other was only positive for anti-HBc. Prevalence of occult HBV was 0.11%.

Conclusion: The incidence rate of HBV was much lower than that of hepatitis C virus (HCV) in the same cohort. We supposed that the discrepancy between incidence rate of HBV and that of HCV was caused by the difference of their carrier rates and of their characteristics for persistent infection. So, we concluded that it is prerequisite to grasp the burden of HBV carriers in the group to prevent new HBV infections in HD patients.

Key words: hemodialysis, hepatitis B virus, incidence rate, occult hepatitis B virus, prevalence

INTRODUCTION

HEPATITIS B VIRUS (HBV) is highly associated with chronic liver disease in Japan. The prevalence of hepatitis B surface antigen (HBsAg) in younger Japanese has markedly improved under national measures to prevent infection of newborn babies from HBV-infected mothers since 1986.[1] Problems remain to be solved in high-risk groups such as homosexual individuals, i.v. drug abusers, medical workers and patients who require invasive treatment, particularly hemodialysis (HD)[2] who have a low acquisition rate of protective antibody with HB vaccination.[3]

During 1999–2003, we conducted a prospective study among HD patients for hepatitis C virus (HCV) infection and revealed the prevalence and incidence of HCV infection among HD patients in Hiroshima, Japan.[4]

In this study, we aimed to determine the prevalence of HBsAg and the incidence rate of HBV infection in patients on HD who are presumed to be at higher risk for HBV infection, to determine the current status of HBV infection, and to recommend prophylactic measures against HBV infection in patients on HD.

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Furthermore, we calculated the positive rate of occult HBV among the HD patients.

METHODS

Study design and serum samples from patients

A PROSPECTIVE COHORT study for hepatitis viral infections among the HD patients was conducted from 1999 to 2003 in nine dialysis centers in Hiroshima Prefecture, Japan. The cohort of the present study includes the 1860 patients who were registered during the 6 months from November 1999 to May 2000, and the survey lasted for 3 years and 3 months, from November 1999 to February 2003 (Fig. 1a). Serum samples from registered patients were gathered from nine hospitals every 3 months through routine blood tests for cell counts and biochemistry. HD patients who provided informed consent took part in this survey.

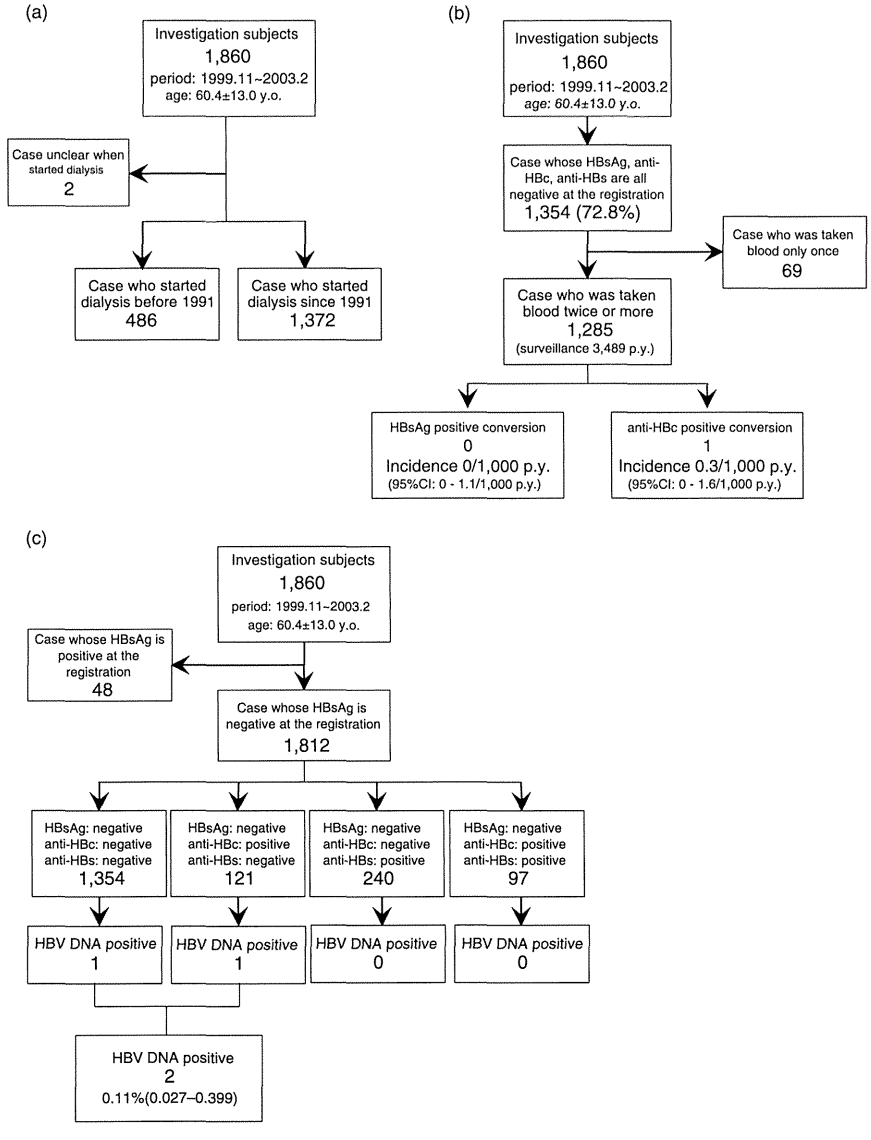

Figure 1 Study designs. (a) Classified by dialysis introduction date. (b) Study for the incidence of hepatitis B virus (HBV) infection. (c) Study for the prevalence of occult HBV infection. CI, confidence interval; HBc, hepatitis B core; HBs, hepatitis B surface; HBsAg, hepatitis B surface antigen.

This study was approved by the ethics committee for epidemiological research of Hiroshima University in Japan.

Incidence of HBV

Among 1860 patients, 1354 were all negative for HBsAg, anti-hepatitis B core (HBc) and anti-hepatitis B surface (HBs) at the start of the survey. Among them, the blood of 1285 patients was taken more than twice (Fig. 1). The patients who became HBsAg positive or anti-HBc positive at the end of the survey were defined as "incidence cases of HBV". Then, the incidence rate was calculated in person-years. In addition, the sera of all the patients who became anti-HBc positive were tested with simultaneous assays for HBsAg, anti-HBc, anti-HBs and HBV DNA.

Prevalence of occult HBV

In the total of 1860 patients, sera of 1812 patients who were negative for HBsAg at the registration were tested for HBV DNA. The sera were pooled into samples, each of which consisted of 20 patients, and were tested for HBV DNA using real-time polymerase chain reaction (PCR). For samples positive for HBV DNA, real-time PCR was then performed on each of the 20 individual serum samples included in the pooled sample, and the samples positive for HBV DNA were identified. When HBV DNA was detected, nucleic acid testing (NAT) via real-time PCR was performed at each point of the survey.

Serological tests

Hepatitis B surface antigen, anti-HBs and anti-HBc were determined using the hemagglutination method. HBsAg was determined by reversed-passive hemagglutination (R-PHA), anti-HBs by passive hemagglutination (PHA) and anti-HBc by inhibition of hemagglutination (HI) using assay kits provided by the Japanese Red Cross Blood Center (JRC).

Sera were diluted twofold serially on a microtiter plate, and the results were assessed by the highest twofold (2^N) dilution of sera that induced or inhibited hemagglutination. The threshold for positive hemagglutination results was 2^2 or more for HBsAg by R-PHA, 2^4 or more for anti-HBs by PHA and 2^5 or more for anti-HBc by HI.

Qualitative assays of HBV DNA were carried out using PCR with nested primers deduced from the S gene sequence. Quantitative assays of HBV DNA were conducted using real-time PCR with Applied Biosystems Step One Real Time PCR System (Life Technologies Japan, Tokyo, Japan) with primers and probe from the S gene. Primers were HBSF2 and HBSR2, and the probe was HBSP2.[5]

Statistical analysis

Prevalence of HBV markers and their 95% confidence interval (CI) were calculated. Differences in the prevalence among groups classified by sex and age were evaluated by the χ^2-test or Fisher's exact test. Risk factors associated with prevalence of HBV markers were determined by multivariate logistic regression analyses. *P*-values of less than 0.05 were considered statistically significant. Statistical analyses were performed with JMP version 9 (SAS Institute, Cary, NC, USA).

RESULTS

Prevalence of HBV infection

IN THIS STUDY, 1860 HD patients were registered, 1108 of whom were men and 752 women, with an average age at registration of 60.4 ± 13.0 years (range, 20–94).

The prevalence of HBsAg among the 1860 patients at registration was 2.6% (95% CI, 1.86–3.30%), anti-HBc positive rate 20.6% (95% CI, 18.81–22.48%) and anti-HBs positive rate 11.7% (95% CI, 10.26–13.18%) (Table 1a). Regarding age distribution, the HBsAg positive rate of 4.2% was highest in the patients in their 50s. Regarding sex, the HBsAg positive rate was 2.8% in men and 2.3% in women, showing the highest rate in patients in their 50s, and the second highest rate in those in their 40s in both men and women. The prevalence of anti-HBc was 20.6% (95% CI, 18.8–22.5%), and the prevalence of anti-HBc was higher in men than women with no significant difference. The prevalence of anti-HBs was 11.7% (95% CI, 10.3–13.2%) and the prevalence of anti-HBs was higher in women than men with no significant difference.

To compare HBV infection with the time of introduction of HD, the patients were divided into two groups: 1372 patients (829 men and 543 women, aged 61.89 ± 13.38 years; range, 20–94) who had started HD since 1991; and 486 patients (279 men and 207 women, aged 56.24 ± 10.64 years; range, 25–93) who started before 1991 (unclear for two patients) (when erythropoietin was approved by the health insurance policy in Japan and transfusions have rarely been used to treat anemia since then) (Figs 1,2, Tables 2,3). The prevalence of HBsAg among the 1372 patients who had started HD since 1991 was 2.1% (95% CI, 1.4–2.9%) and that of HBsAg among 486 patients who started before 1991 was 3.9% (95% CI, 2.2–5.6%). Prevalence of anti-HBc was higher among the patients who started before 1991 than the patients who

Table 1 Prevalence of HBsAg, anti-HBc, and anti-HBs stratified by age and sex in 1860 registered patients

Age at registration (years)	Total			Male			Female		
	n	Positive (%)	95% CI	*n*	Positive (%)	95% CI	*n*	Positive (%)	95% CI
					HBsAg				
≤39	105	2 (1.9)	0–4.52	76	1 (1.3)	0–3.88	29	1 (3.4)	0–10.09
40–49	255	9 (3.5)	1.26–5.80	162	6 (3.7)	0.80–6.61	93	3 (3.2)	0–6.82
50–59	528	22 (4.2)	2.46–5.87	330	15 (4.5)	2.30–6.80	198	7 (3.5)	0.96–6.11
60–69	491	11 (2.2)	0.93–3.55	270	7 (2.6)	0.70–4.49	221	4 (1.8)	0.05–3.57
≥70	481	4 (0.8)	0.02–1.64	270	2 (0.7)	0–1.76	211	2 (0.9)	0–2.15
Total	1,860	48 (2.6)	1.86–3.30	1,108	31 (2.8)	1.83–3.77	752	17 (2.3)	1.20–3.32
					Anti-HBc				
≤39	105	12 (11.4)	5.34–17.51	76	9 (11.8)	4.58–19.10	29	3 (10.3)	0–21.43
40–49	255	47 (18.4)	13.67–23.19	162	33 (20.4)	14.17–26.57	93	14 (15.1)	7.79–22.32
50–59	528	128 (24.2)	20.59–27.90	330	93 (28.2)	23.33–33.04	198	35 (17.7)	12.36–23.00
60–69	491	91 (18.5)	15.10–21.97	270	50 (18.5)	13.89–23.15	221	41 (18.6)	13.43–23.68
≥70	481	106 (22.0)	18.33–25.74	270	57 (21.1)	16.24–25.98	211	49 (23.2)	17.52–28.92
Total	1,860	384 (20.6)	18.81–22.48	1,108	242 (21.8)	19.40–24.27	752	142 (18.9)	16.09–21.68
					Anti-HBs				
≤39	105	6 (5.7)	1.27–10.15	76	2 (2.6)	0–6.23	29	4 (13.8)	1.24–26.34
40–49	255	32 (12.5)	8.48–16.62	162	22 (13.6)	8.30–18.86	93	10 (10.8)	4.46–17.05
50–59	528	64 (12.1)	9.33–14.91	330	35 (10.6)	7.28–13.93	198	29 (14.6)	9.72–19.57
60–69	491	57 (11.6)	8.78–14.44	270	32 (11.9)	8.00–15.71	221	25 (11.3)	7.14–15.49
≥70	481	59 (12.3)	9.33–15.20	270	36 (13.3)	9.28–17.39	211	23 (10.9)	6.70–15.11
Total	1,860	218 (11.7)	10.26–13.18	1,108	127 (11.5)	9.59–13.33	752	91 (12.1)	9.78–14.43

CI, confidence interval; HBc, hepatitis B core; HBs, hepatitis B surface; HBsAg, hepatitis B surface antigen.

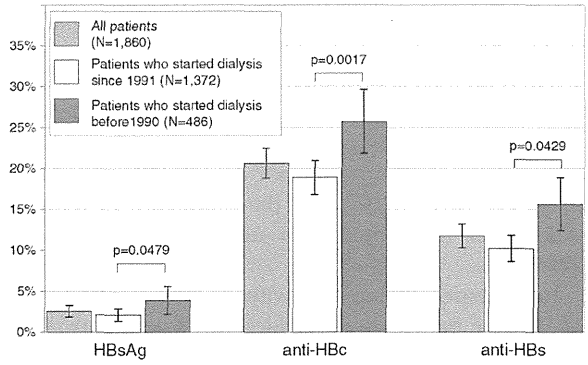

Figure 2 Prevalence of hepatitis B virus (HBV) markers classified by dialysis introduction date. HBc, hepatitis B core; HBs, hepatitis B surface; HBsAg, hepatitis B surface antigen.

started since 1991 (25.5%; 95% CI, 21.6–29.3% vs 18.9%; 95% CI, 16.8–21.0%) with significant difference ($P < 0.01$).

Prevalence of anti-HBs among the patients who started before 1991 was higher than among the patients who started since 1991 ($P = 0.0429$) (16.4%; 95% CI, 12.8–19.3% vs 10.2%; 95% CI, 8.6–11.8%).

Among the 1372 patients who started since 1991, the results of HBV infection were compared by duration on HD. The prevalence of HBsAg was the highest in patients who were on HD for over 7 years (4.7%). Likewise, anti-HBc and anti-HBs were the highest among the patients who were on HD for 7 years or more. With respect to sex, in men, the prevalence of HBsAg and anti-HBc positive rates were the highest in those who had been on HD for 7 years or more, at 7.2% and 24.6%, respectively, and the anti-HBs positive rate was the highest (14%) in men who had been on HD for 5–6 years. In women, the prevalence of HBsAg was the highest (3.8%) among the patients who had been on HD for 3–4 years. Anti-HBc positive rate and anti-HBs positive rate were the highest in women who had been on HD for 7 years or more, at 25.9% and 22.4%, respectively.

Among the 1372 patients, risk factors associated with prevalence of HBV markers were determined by multivariate logistic regression analyses (Table 4). The prevalence of HBsAg in patients aged 70 years or more old is significantly lower than that in patients aged 49 years or less. There were no significant differences with respect to sex and duration of HD. Anti-HBc positive rate was significantly higher than that of patients aged 49 years or less, and the prevalence of

Table 2 Prevalence of HBsAg, anti-HBc, and anti-HBs stratified by age and sex in 1372 patients who started hemodialysis since 1991

Age at registration (years)	Total			Male			Female		
	n	Positive (%)	95% CI	*n*	Positive (%)	95% CI	*n*	Positive (%)	95% CI
					HBsAg				
≤39	77	1 (1.3)	0–3.83	55	1 (1.8)	0–5.35	22	0 0.0	0–16.77
40–49	156	6 (3.8)	0.83–6.86	99	5 (5.1)	0.74–9.36	57	1 (1.8)	0–5.16
50–59	340	12 (3.5)	1.57–5.49	213	8 (3.8)	1.20–6.31	127	4 (3.1)	0.11–6.19
60–69	371	7 (1.9)	0.50–3.27	214	4 (1.9)	0.06–3.68	157	3 (1.9)	0–4.05
≥70	428	3 (0.7)	0–1.49	248	1 (0.4)	0–1.19	180	2 (1.1)	0–2.64
Total	1372	29 (2.1)	1.35–2.87	829	19 (2.3)	1.27–3.31	543	10 (1.8)	0.71–2.97
					Anti-HBc				
≤39	77	7 (9.1)	2.67–15.51	55	6 (10.9)	2.67–19.15	22	1 (4.5)	0–13.3
40–49	156	21 (13.5)	8.11–18.82	99	13 (13.1)	6.48–19.78	57	8 (14.0)	5.02–23.05
50–59	340	69 (20.3)	16.02–24.57	213	49 (23.0)	17.35–28.66	127	20 (15.7)	9.41–22.08
60–69	371	68 (18.3)	14.39–22.27	214	40 (18.7)	13.47–23.92	157	28 (17.8)	11.85–23.82
≥70	428	94 (22.0)	18.04–25.89	248	52 (21.0)	15.90–26.03	180	42 (23.3)	17.15–29.51
Total	1372	259 (18.9)	16.81–20.95	829	160 (19.3)	16.61–21.99	543	99 (18.2)	14.98–21.48
					Anti-HBs				
≤39	77	4 (5.2)	0.24–10.15	55	1 (1.8)	0–5.35	22	3 (13.6)	0–27.98
40–49	156	16 (10.3)	5.49–15.02	99	11 (11.1)	4.92–17.30	57	5 (8.8)	1.43–16.12
50–59	340	33 (9.7)	6.56–12.85	213	15 (7.0)	3.61–10.48	127	18 (14.2)	8.11–20.24
60–69	371	34 (9.2)	6.23–12.10	214	21 (9.8)	5.83–13.80	157	13 (8.3)	3.97–12.59
≥70	428	53 (12.4)	9.26–15.50	248	33 (13.3)	9.08–17.53	180	20 (11.1)	6.52–15.70
Total	1372	140 (10.2)	8.60–11.81	829	81 (9.8)	7.75–11.79	543	59 (10.9)	8.25–13.48

CI, confidence interval; HBc, hepatitis B core; HBs, hepatitis B surface; HBsAg, hepatitis B surface antigen.

anti-HBs in patients who had been on HD for 5 years or more was significantly higher than that of patients on HD for less than 1 year.

Among the 1860 patients, risk factors associated with prevalence of HBV markers were determined by multivariate logistic regression analyses (Table 5). Risk of anti-HBs and anti-HBc are higher significantly in the group who started HD before 1991 than in the group since 1991, but as for the risk of HBsAg, there is no significant difference between groups.

Incidence rate of HBV infection

The subjects were 1285 patients whose samples were taken more than twice and who were negative for HBsAg, anti-HBc and anti-HBs at the start of the survey (Fig. 1b). There was no case of conversion to HBsAg positivity during the survey; hence, the incidence rate of HBV infection as determined by conversion to HBsAg positivity was 0/1000 person-years (95% CI, 0–1.1/1000 person-years). On the other hand, there was one case of positive conversion to anti-HBc; hence, the incidence rate of HBV infection as determined by conversion to anti-HBc positivity was 0.3/1000 person-years (95% CI, 0–1.6/1000 person-years).

In the patient who converted to anti-HBc positivity, anti-HBc titer increased from 2^2 to 2^7 on the third examination and peaked at 2^8 on the fourth examination. Only then, when the titer was at its peak, was HBV DNA detected. Subsequently, HBV DNA was not detected (Fig. 3). This patient was a 73-year-old man who was affected by diabetic nephropathy and was on HD for 2 months before the registration, then died in May 2001 (after the seventh survey).

Prevalence of occult HBV at the registration

Among the 1812 patients who were negative for HBsAg at the start of the survey (Fig. 1c), one occult HBV infection out of 1354 patients who were negative for HBsAg, anti-HBc and anti-HBs, and one occult HBV infection out of 121 patients who were negative for both HBsAg and anti-HBs and positive for anti-HBc were determined to be HBV DNA positive. That is, two out of 1812 patients were positive for HBV DNA, and the prevalence of occult HBV rate was 0.11% (95% CI, 0.027–0.399%). Of two occult HBV infections, one was a 67-year-old

Table 3 Prevalence of HBsAg, anti-HBc and anti-HBs stratified by age and sex in 486 patients who started hemodialysis before 1991

Age at registration (years)	Total			Male			Female		
	n	Positive (%)	95% CI	*n*	Positive (%)	95% CI	*n*	Positive (%)	95% CI
					HBsAg				
≤39	28	1 (3.6)	0–10.45	21	0 (0.0)	0–17.57	7	1 (14.3)	0–40.21
40–49	99	3 (3.0)	0–6.41	63	1 (1.6)	0–4.67	36	2 (5.6)	0–13.04
50–59	188	10 (5.3)	2.11–8.53	117	7 (6.0)	1.69–10.28	71	3 (4.2)	0–8.90
60–69	120	4 (3.3)	0.12–6.55	56	3 (5.4)	0–11.25	64	1 (1.6)	0–4.60
≥70	51	1 (2.0)	0–5.77	22	1 (4.5)	0–13.25	29	0 (0.0)	0–12.72
Total	486	19 (3.9)	2.19–5.63	279	12 (4.3)	1.92–6.68	207	7 (3.4)	0.92–5.84
					Anti-HBc				
≤39	28	5 (17.9)	3.67–32.04	21	3 (14.3)	0–29.25	7	2 (28.6)	0–62.04
40–49	99	26 (26.3)	17.59–34.93	63	20 (31.7)	20.25–43.24	36	6 (16.7)	4.49–28.84
50–59	188	59 (31.4)	24.75–38.02	117	44 (37.6)	28.83–46.38	71	15 (21.1)	11.63–30.62
60–69	120	23 (19.2)	12.12–26.21	56	10 (17.9)	7.83–27.89	64	13 (20.3)	10.46–30.17
≥70	51	12 (23.5)	11.89–35.17	22	5 (22.7)	5.22–40.24	29	7 (24.1)	8.56–39.71
Total	486	125 (25.7)	21.89–29.61	279	82 (29.4)	24.05–34.74	207	43 (20.8)	15.25–26.30
					Anti-HBs				
≤39	28	2 (7.1)	0–16.68	21	1 (4.8)	0–13.87	7	1 (14.3)	0–40.21
40–49	99	16 (16.2)	8.91–23.41	63	11 (17.5)	8.09–26.83	36	5 (13.9)	2.59–25.19
50–59	188	31 (16.5)	11.18–21.79	117	20 (17.1)	10.27–23.92	71	11 (15.5)	7.08–23.91
60–69	120	23 (19.2)	12.12–26.21	56	11 (19.6)	9.24–30.05	64	12 (18.8)	9.19–28.31
≥70	51	4 (7.8)	0.46–15.22	22	3 (13.6)	0–27.98	29	1 (3.4)	0–10.09
Total	486	76 (15.6)	12.41–18.87	279	46 (16.5)	12.13–20.84	207	30 (14.5)	9.70–19.29

CI, confidence interval; HBc, hepatitis B core; HBs, hepatitis B surface; HBsAg, hepatitis B surface antigen.

Table 4 4aOutput of logistic regression analyses (at registration) ($n = 1372$)

		HBsAg			Anti-HBc			Anti-HBs		
		OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Sex	*n*									
Female (Ref.)	543	1.00			1.00			1.00		
Male	829	1.23	(0.57–2.78)	0.609	1.11	(0.84–1.47)	0.474	0.92	(0.64–1.31)	0.631
Age (years)	*n*									
≤49 (Ref.)	233	1.00			1.00			1.00		
50-69	711	0.89	(0.38–2.30)	0.789	1.74	(1.14–2.74)	0.013	1.08	(0.65–1.88)	0.762
≥70	428	0.23	(0.05–0.85)	0.037	2.07	(1.33–3.33)	0.001	1.53	(0.90–2.68)	0.127
Dialysis period	*n*									
<1 (Ref.)	383	1.00			1.00			1.00		
1–4	684	1.06	(0.44–2.84)	0.895	0.94	(0.68–1.30)	0.707	1.24	(0.80–1.94)	0.345
5–8	305	1.34	(0.47–3.88)	0.578	1.09	(0.74–1.60)	0.646	1.71	(1.04–2.82)	0.033

CI, confidence interval; HBc, hepatitis B core; HBs, hepatitis B surface; HBsAg, hepatitis B surface antigen; OR, odds ratio; Ref., reference.

woman who was affected by diabetic mellitus and on HD for 7 months before the registration. She died after taking blood samples at the start of the survey, and the status of her HBV markers is unclear. The quantity of HBV DNA was 9.87×10^4 copies/mL. The other patient was a 68-year-old woman who was affected by diabetic nephropathy and on HD for 6 years before the registration. During the survey, neither HBsAg nor anti-HBs was detected, and only anti-HBc remained positive from the start of the survey. The quantity of HBV DNA fluctuated between 6.00×10^1 copies/mL and 3.00×10^3 copies/mL.

Table 5 4bOutput of logistic regression analyses (at registration) ($n = 1860$)

		HBsAg			Anti-HBc			Anti-HBs		
		OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Sex	*n*									
Female (Ref.)	752	1.00			1.00			1.00		
Male	1108	1.15	(0.59–2.29)	0.688	1.36	(1.07–1.72)	0.011	0.97	(0.73–1.29)	0.843
Age (years)	*n*									
≤49 (Ref.)	360	1.00			1.00			1.00		
50–69	1019	1.29	(0.61–3.06)	0.523	1.51	(1.10–2.09)	0.010	1.32	(0.91–1.96)	0.151
≥70	481	0.21	(0.03–0.86)	0.028	1.79	(1.25–2.59)	0.002	1.65	(1.07–2.58)	0.024
Introduced to dialysis	*n*									
Since 1991 (Ref.)	1372	1.00			1.00			1.00		
Before 1991	486	1.19	(0.58–2.32)	0.625	1.55	(1.20–2.00)	0.001	1.83	(1.35–2.46)	<0.001

CI, confidence interval; HBc, hepatitis B core; HBs, hepatitis B surface; HBsAg, hepatitis B surface antigen; OR, odds ratio; Ref., reference.

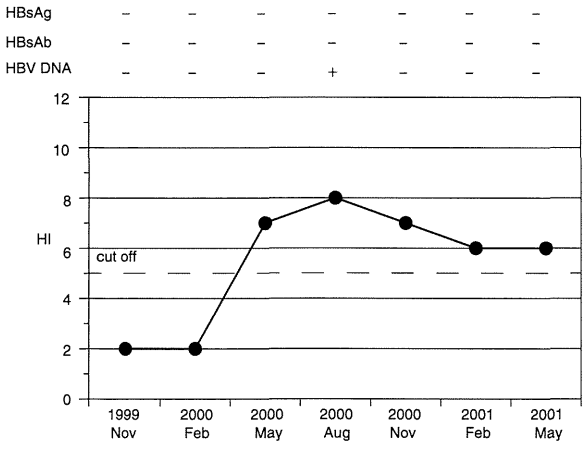

Figure 3 Changes of hepatitis B virus (HBV) markers in the those who converted to anti-hepatitis B core (HBc) positive status, implying new infection.

DISCUSSION

THE RISKS OF exposure to hepatitis viruses are high in HD patients, including errors made by staff during medical procedures, exposure to blood via dialysis instruments and contamination of equipment.[6] Transfusion therapy was one of the major infection routes of hepatitis viruses before screening tests for hepatitis viruses were established at blood centers.[7] This was especially true for HD patients[8,9] who had to receive frequent transfusion therapy to treat nephrogenic anemia, a complication of kidney disturbance. Since 1991, when erythropoietin was approved by the health insurance policy in Japan, transfusions have rarely been used to treat anemia. Therefore, the risk of HBV and HCV infection due to transfusion has decreased substantially.

In this survey, the prevalence of HBsAg among 1860 patients including those who started HD from 1969 was 2.6%. But to evaluate the risk of HBV infection excluding the effect of blood transfusion, we investigated the 1372 patients who had started HD since 1991 when erythropoietin was approved by the health insurance policy in Japan, and the 486 patients before 1991. The prevalence of HBsAg among the 1372 patients who started HD since 1991 was 2.1%. On the other hand, the prevalence of HBsAg among the 486 patients who started before 1991 was 3.9%. The prevalence since 1991 was slightly lower than that before 1991, presumably due to the decreased risk of HBV infection from transfusion ($P = 0.048$) in univariate analysis and moreover by the logistic regression analysis; as for the risk of HBsAg, there is no significant difference in those who had started since 1991 and those before 1991.

The reported HBsAg positive rate was 2.4% according to a cross-sectional survey of 92 460 HD patients in 2006.[10] Another survey of 30 916 patients who underwent surgery in regional center hospitals in Hiroshima Prefecture between 1993 and 2001 reported that the prevalence of HBsAg was 1.8%.[11] Each of the studies, including this survey, showed higher prevalence of HBsAg among HD patients than that among first-time donors and the general population in Japan in medical investigations of hepatitis viruses (0.63%).[1] However, the prevalence of HBsAg at 2.1% among the 1372 patients who were introduced HD since 1991 is not much higher than that of the general population, particularly considering that Hiroshima Prefecture is an area with a high prevalence of HBsAg and the prevalence of HBsAg in first-time blood donors who were the same age as those in this study was 2.1% in the Chugoku district in 2000.[12]

Anti-HBc positive rate among 1372 patients who started HD since 1991 in this study indicated that the rate of exposure to HBV infection was 18.9%, but the anti-HBc positive rate among 486 patients who started before 1991 was 25.5%. Among the 486 patients, anti-HBc positive rate was higher than that among the patients who started HD treatment since 1991 ($P < 0.01$). In addition, by the logistic regression analysis of the risk of anti-HBc and anti-HBs, there is significant difference between "since 1991" and "before 1991". We considered that the risk of HBV infection is higher in the group who started HD before 1991 than since 1991.

The pilot survey was conducted in the same prefecture among 1637 company workers who had received health check-ups (mean age, 49.3 ± 14.9 years old; range, 19–81; unpublished data), and the anti-HBc positive rate was 18.0%, which is the same as among the HD patients obtained in this study.

This result showed that the risk of HBV infection among HD patients was not so significantly high and that HD patients may not produce anti-HBc sufficiently.[13]

Multivariate analysis indicated that the risk of being positive for HBsAg did not correlate with the duration of HD, but did correlate with age of 70 years or more. The younger generation has a significantly higher risk of persistent HBV infection ($P = 0.037$), but the older generation has high prevalence of anti-HBc (HBV exposure rate) and anti-HBs. Thus, these results show that the lower HBsAg positive rate with older age may be attributed to loss of HBsAg.

The prevalence of anti-HBs in female patients was slightly higher than that in male patients without statistical significance and that of HBsAg and anti-HBc in female patients was lower than male patients. We need to study spontaneous HBV clearance and the loss of HBsAg by sex.

The risk of anti-HBc positivity was twofold higher in patients aged 70 years or more. A significant risk factor for conversion to anti-HBs positivity was duration of HD of 5 years or more (range, 5–8). This finding suggests that exposure to HBV infection occurs in proportion to the length of time on HD.

In our study, the incidence rate of HBsAg was 0/1000 person-years (95% CI, 0–1.1/1000 person-years) and the incidence rate of HBV infection determined by conversion to anti-HBc positivity was 0.3/1000 person-years (95% CI, 0–1.6/1000 person-years). Thus, the incidence rate of HBV infection in HD patients would be slightly higher than that of first-time blood donors ($2.78/10^5$ person-years; 95% CI, $1.78–4.14/10^5$ person-years).[14] The observed incidence rate of HBV infection, determined by the conversion to HBsAg positivity and/or anti-HBc positivity, is much lower than the incidence rate of HCV infection in the same cohort of patients during the same observation period, at $330/10^5$ person-years. The difference may be attributed to the difference in carrier rates; while the HBV carrier rate in the group was 2.1% at the start of the survey, the HCV carrier rate is as high as 15.7% in the same cohort.[4] Such a wide difference between HCV and HBV could be due to the size of reservoir, which would be far larger for HCV than HBV in HD patients.[15] HBV infection resolves in most cases and rarely persists. Unlike a HCV carrier, a patient infected with HBV can seldom serve as a reservoir for further spread of HBV. We supposed that the discrepancy between incidence rate of HBV and that of HCV was caused by the difference of their carrier rates and of their characteristics for persistent infection. This fact could explain why the incidence of HBV is low. When designing and implementing preventive measures in a given HD center, it should be prerequisite to determine and take account of the size of the population of patients with persistent HBV and HCV.[16]

Hepatitis B virus DNA was examined to investigate occult HBV in HD patients who were negative for HBsAg, and two HBV DNA positive cases were detected. Neither case was in the early phase of infection. In one case, a blood sample was taken only once at registration, and further analysis could not be done because of their death. In the other case, anti-HBc was positive and HBsAg was negative, indicating the state of late phase of HBV infection or occult HBV status. HBsAg tests in general clinics show negative results in such cases because the quantity of antigen is below the limit of detection. These cases are thus regarded as "no existence of HBV" in the clinics. In this study, the prevalence of occult HBV of 0.11% (95% CI, 0.03–0.40%) was not so high.[17] We detected HBV DNA by real-time PCR in 20 samples via pooled NAT to find positive samples at first because we confirmed that we could detect over 10^1 copies/mL by real-time PCR and HBV DNA could be detected more than cut-off 10^2 copies/mL by pooled NAT. But there is a possibility that the prevalence may be underestimated by pooled NAT compared with individual NAT. In addition, serological tests were done using assay kits provided by the JRC, which is not more sensitive than other kits such as chemiluminescent immunoassay.

In this study, the prevalence of HBsAg and incidence rate of HBV infection were lower than usually expected in HD patients. Furthermore, the risk of new HBV infection did not increase during the course of HD. The HD centers participating in this study have been using a nosocomial infection prevention program to prevent HCV infection since 1999.[4] The low incidence rate of HBV infection observed in this study seems to be one of the effects of this program.

Western guidelines such as KDIGO (Kidney Disease: Improving Global Outcomes)[18] have established that HD patients should be vaccinated against HBV. However, it has been pointed out that HB vaccination in HD patients is not common in Japan despite this recommendation,[5] as observed in the subjects of this study. Changes in the HB vaccination policy in Japan may enable the rate of HBV infection in HD patients to approach zero.

To prevent new HBV infections in HD patients, it is prerequisite to grasp the size of HBV carriers in the group and make strategies for prevention.

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Short Communication

Seroprevalence, genotypic distribution and potential risk factors of hepatitis B and C virus infections among adults in Siem Reap, Cambodia

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Aim: We investigated hepatitis B virus (HBV) and hepatitis C virus (HCV) infections among adults in Siem Reap, Cambodia, to consider the prevention strategy in cooperation with the Ministry of Health in Cambodia.

Methods: Serological tests for determining HBV and HCV infections and questionnaires were performed from 2010 to 2012 among the general population in the province of Siem Reap. Multivariate logistic regression analysis was conducted to clarify the factors related to HBV and HCV infections.

Results: There were 483 participants, comprising 194 men and 289 women (age range, 18–89 years). The prevalence of hepatitis B surface antigen was not very high at 4.6%, while anti-hepatitis B core (anti-HBc) was high at 38.5%. All HBV DNA samples were classified as genotype C. Anti-HBc showed the trend that the older the age, the higher the positive rate ($P = 0.0002$). The prevalence of HCV RNA and anti-HCV were 2.3% and 5.8%, respectively. HCV RNA was detected in 39.3% of anti-HCV positive samples and most of them were classified as genotype 6 (54.5%) and 1 (27.3%). Remarkably, in multivariate logistic regression analysis, history of operation and blood transfusion were significantly associated with the positivity for HBV infection and HCV RNA, respectively.

Conclusion: Our results showed that operation and blood transfusion were potential risk factors for HBV and HCV infection, respectively, and supposed that horizontal HBV transmission may be frequent in adults in Cambodia. Hence, for reducing HBV and HCV infections, it is necessary to improve the safety of blood and medical treatment.

Key words: adults, Cambodia, hepatitis B virus, hepatitis C virus, seroepidemiology

INTRODUCTION

HEPATITIS B VIRUS (HBV) and hepatitis C virus (HCV) infections are serious problems globally. Approximately 57% of cases of liver cirrhosis and 78% of cases of primary liver cancer result from HBV or HCV infection.[1] Worldwide, more than 2 billion people have been infected with HBV,[1,2] and 240–350 million have chronic infections,[3,4] while approximately 150 million people are chronically HCV infected.[5]

In the Western Pacific Region, the World Health Organization has estimated that there is 160 million people with chronic HBV infections.[1] Most countries in the region have an estimated rate of 1–2% for HCV infection.[1,6]

In Cambodia, one of the countries in the Western Pacific Region, the prevalence of HBV and HCV infections is assumed to be high. Furthermore, according to the GLOBOCAN 2012, liver cancer was the most common cause of cancer death with the highest mortality of 21.5/100 000.[7] However, few data is available about HBV and HCV infections among the general

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population in Cambodia. Therefore, we investigated the HBV and HCV infections for seroprevalence, genotypic distribution and risk factors among adults in the general population of Cambodia, for planning a preventive strategy in cooperation with the Ministry of Health in Cambodia.

METHODS

Subjects

OUR SERO-EPIDEMIOLOGICAL STUDY was performed from 2010 to 2012 among the general population in three villages/communes in Siem Reap, a province located in northwestern Cambodia with a population of 896 443.[8] The subjects were over the age of 18 years, who are considered as adults in Cambodia.

All the residents in the village/commune were called to participate in this study by village/commune chiefs. Duty officers of the Ministry of Health in Cambodia explained the study protocol to the participants before they were enrolled in our study.

Ethical issues

This study was approved by the ethics committee for epidemiological research of Hiroshima University in Japan and that of the Ministry of Health in Cambodia. We conducted the survey after obtaining written informed consents from all participants.

Serological tests

Approximately 10 mL of blood samples taken from participants were carefully centrifuged, and sera were brought to Hiroshima University in Japan and tested for hepatitis virus markers to determine the HBV and HCV infections by the following methods.

For HBV infection, hepatitis B surface antigen (HBsAg) was determined by reversed passive hemagglutination assay (R-PHA). Passive hemagglutination (PHA) or chemiluminescence immunoassay (CLIA) was used to detect hepatitis B surface antibody (anti-HBs). Hepatitis B core antibody (anti-HBc) was detected by PHA or CLIA. HBV DNA was determined by real-time polymerase chain reaction (PCR) with primers deduced from HBV surface gene. HBV genotyping was performed by either enzyme immunoassay or direct sequencing. HBV infection was defined as the seropositivity for HBsAg and/or anti-HBc.

For HCV infection, sera were tested for HCV antibody (anti-HCV) by particle agglutination test (PA). HCV RNA was detected by real-time PCR with primers deduced from the conserved region in the 5′-non-coding region of the viral genome. HCV infection was defined as the seropositivity for anti-HCV.

Questionnaires

Questionnaire consisted of the basic background (sex, age, occupation) and eight questions including current health status, current periodic treatment, history of disease or a major injury, history of injection or infusion, operation, blood transfusion, tattoo and holes for pierced earrings.

Statistical analysis

All data were analyzed using JMP version 9 (SAS Institute, Cary, NC, USA). The proportions were estimated with the 95% confidence interval (CI). The χ^2-test or Fisher's exact test and Mantel extension test for trend were performed to evaluate the difference in the prevalence of hepatitis viral markers by sex, age and residence. Univariate analysis using the χ^2-test or Fisher's exact test and multivariate logistic regression analysis using a stepwise selection method were performed to identify the potential risk factors (sex, age groups and eight questions) related to HBV and HCV infections by calculating odds ratio (OR) and 95% CI. Multivariate logistic regression analysis was conducted in which terms were retained if they reached the 0.25 level of significance. Factors selected by the stepwise selection method and age groups were included in a multivariate model. For all analyses, $P < 0.05$ was considered statistically significant.

RESULTS

TOTAL PARTICIPANTS WERE 483 adults, comprising 194 men (40.2%) and 289 women (59.8%). Ages ranged 18–89 years as of 2013, and the average age was 40.7 ± 14.7 years.

According to the results of questionnaires, most participants (301/483; 62.3%) answered that they did not feel healthy at present. For medical history, 41.2% (199/483) of them answered that in the past they had disease or major injury. Most participants had received treatment by injection or infusion (369/483; 76.4%), while fewer participants had had an operation (46/483; 9.5%) or blood transfusion (5/444; 1.1%).

In this study, among 483 Cambodian adults, the prevalence of HBsAg, anti-HBs and anti-HBc was 4.6% (95% CI, 2.7–6.4%), 30.2% (26.1–34.3%) and 38.5% (34.2–42.8%), respectively (Table 1). The prevalence of anti-HBc was significantly higher in men than in

Table 1 Prevalence of HBsAg, anti-HBs, anti-HBc, anti-HCV and HCV RNA among the general population in Siem Reap, Cambodia

		n	HBsAg positive			Anti-HBs positive			Anti-HBc positive			HCV RNA positive			Anti-HCV positive		
			n	(%)	(95% CI)	*n*	(%)	(95% CI)	*P*†	*n*	(%)	(95% CI)	*P*†	*n*	(%)	(95% CI)	*P*†	*n*	(%)	(95% CI)	*P*†
Total		483	22	(4.6)	(2.7–6.4)	146	(30.2)	(26.1–34.3)		186	(38.5)	(34.2–42.8)		11	(2.3)	(0.95–3.6)		28	(5.8)	(3.7–7.9)	
Sex	Male	194	12	(6.2)	(2.8–9.6)	65	(33.5)	(26.9–40.1)	0.1591	87	(44.9)	(37.8–51.8)	0.1988	5	(2.6)	(0.35–4.8)	0.0191*	11	(5.7)	(2.4–8.9)	0.7174
	Female	289	10	(3.5)	(1.4–5.6)	81	(28.0)	(22.8–33.2)		99	(34.3)	(28.8–39.7)		6	(2.1)	(0.43–3.7)		17	(5.9)	(3.2–8.6)	0.9220
Age group (years)	18–29	131	4	(3.1)	(0.11–6.0)	26	(19.9)	(13.0–27.0)	0.3393	32	(24.4)	(17.1–31.8)	0.0003*	1	(0.76)	(0–2.3)	0.0011*	3	(2.3)	(0–4.9)	0.0023*
	30–39	121	9	(7.4)	(2.8–12.1)	29	(24.0)	(16.4–31.6)		47	(38.8)	(30.2–47.5)		1	(0.83)	(0–2.4)		4	(3.3)	(0.12–6.5)	0.0004*
	40–49	103	6	(5.8)	(1.3–10.3)	35	(34.0)	(24.8–43.1)		47	(45.6)	(36.0–55.3)		3	(2.9)	(0–6.2)		6	(5.8)	(1.3–10.3)	
	50–59	66	1	(1.5)	(0–4.5)	31	(47.0)	(34.9–59.0)		34	(51.5)	(39.5–63.6)		0	(0.0)	(0–5.6)		4	(6.1)	(0.30–11.8)	
	60–89	62	2	(3.2)	(0–7.6)	25	(40.3)	(28.1–52.5)		26	(41.9)	(29.7–54.2)		6	(9.7)	(2.3–17.0)		11	(17.7)	(8.2–27.3)	
Residence	KC	186	2	(1.1)	(0–2.6)	35	(18.8)	(13.2–24.4)	0.0096*	47	(25.3)	(19.0–31.5)	<0.0001*	3	(1.6)	(0–3.4)	0.3860	3	(1.6)	(0–3.4)	0.0078*
	CV	249	18	(7.2)	(4.0–10.4)	95	(38.2)	(32.1–44.2)		117	(47.0)	(40.8–53.2)		8	(3.2)	(1.0–5.4)		21	(8.4)	(5.0–11.9)	
	RV	48	2	(4.2)	(0–9.8)	16	(33.3)	(20.0–46.7)		22	(45.8)	(31.7–59.9)		0	(0.0)	(0–0.077)		4	(8.3)	(0.51–16.2)	

†χ^2-Test or Fisher's exact test
*Statistically significant variables.
CI, confidence interval; CV, Chrey village; HBc, hepatitis B core; HBs, hepatitis B surface; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; KC, Krabei Riel commune; RV, Rohal village.

women, while there was no difference between men and women in the HBsAg positive rate. There were significant differences among three residences in HBsAg, anti-HBs and anti-HBc positive rates ($P = 0.0096$, <0.0001 and <0.0001). HBV DNA was detected among all HBsAg positives, and they were classified as genotype C. Moreover, both anti-HBs and anti-HBc showed the trend that the older the age, the higher the positive rate (Mantel extension test for trend; $P = 0.0001$, 0.0002) (Fig. 1a). A total of 164 participants (34.0%), who were HBsAg negative and anti-HBc positive, had previous HBV infection. We found 29 participants (6.0%) who were positive for anti-HBs only, possibly due to vaccination. The proportion of non-infected persons who were negative for all HBV markers was 55.5% (268/483).

For HCV infection, the prevalence of HCV RNA, which means HCV carrier rate, was 2.3% (95% CI, 0.95–3.6%) and that of anti-HCV was 5.8% (95% CI, 3.7–7.9%) (Table 1). HCV RNA was detected in 39.3% of anti-HCV positive samples. Both anti-HCV and HCV RNA positive rates tended to increase with age significantly (Mantel extension test for trend; $P = 0.0004$, 0.023) (Fig. 1b). There was no significant difference between men and women in the prevalence of both HCV RNA and anti-HCV, while anti-HCV positive rates were significantly different among three residences ($P = 0.0078$). Of 11 HCV RNA positive samples, six were classified as genotype 6 (54.5%) (6f [$n = 3$], 6e [$n = 2$], 6s [$n = 1$]), three (27.3%) as genotype 1 (1b) and two (18.2%) were unclassified. The other 17 anti-HCV positives were negative for HCV RNA, indicating previous HCV infection (3.5%).

For HBV infection, in multivariate analysis, men were significantly related to positivity for HBV infection (adjusted OR, 2.0; 95% CI, 1.3–3.1). Compared with the age group of 18–29 years, all the older age groups were significantly associated with positivity for HBV infection in multivariate analysis. Remarkably, history of operation was significantly associated with positivity for markers of HBV infection in multivariate analysis (adjusted OR, 1.9, 95% CI, 1.0–3.7) (Table 2).

Regarding HCV infection, the oldest age group (60–89) was significantly associated with the positivity for HCV RNA and also related to the positivity for anti-HCV in multivariate analysis. Notably, history of blood transfusion was significantly associated with the positivity for HCV RNA in our multivariate analysis (adjusted OR, 30.8; 95% CI, 1.0–575.3) (Table 3).

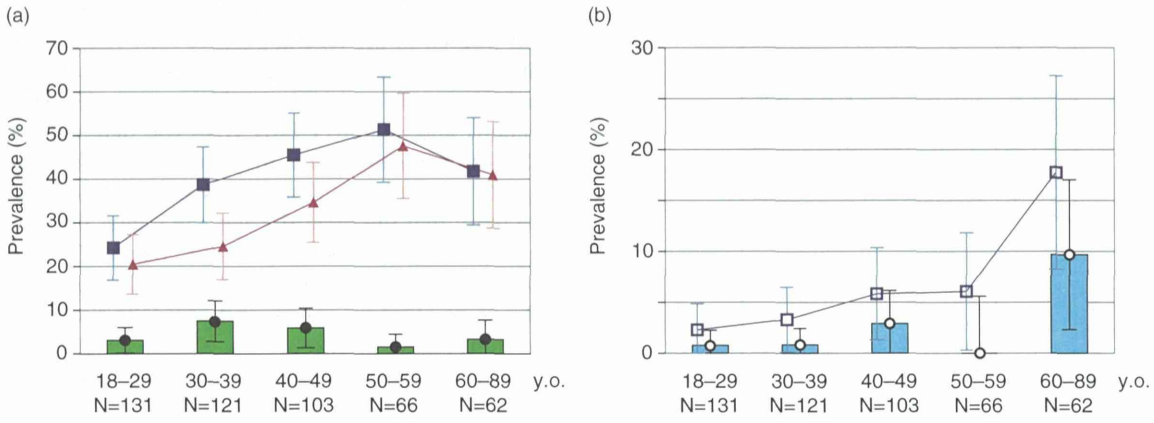

Figure 1 Age-specific prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection markers among the general population in Siem Reap, Cambodia. (a) The prevalence of HBV infection markers including hepatitis B surface antigen (HBsAg), anti-hepatitis B core (anti-HBc) and hepatitis B surface antibody (anti-HBs). (b) The prevalence of HCV infection markers including HCV RNA and anti-HCV. The prevalence of each marker is shown in the line and bar graph. Error bar indicates 95% confidence interval. HBs Ag; anti-HBc; anti-HBs; HCV RNA; anti-HCV.

DISCUSSION

OUR STUDY SHOWED that the HBsAg positive rate was 4.6% ($n = 483$) in adults among the general population in Siem Reap Province, Cambodia, lower than the reported HBsAg seroprevalence of 7.7–10.8%.[9–13] Data from the Cambodia National Blood Transfusion Center in 2012 also showed that the positive rate of HBsAg was 6.3% (3026/48298; 95% CI, 6.05–6.48) among blood donors (Ministry of Health in Cambodia, 2013, unpubl. data). Despite low prevalence of HBsAg, in our study, the prevalence of anti-HBc was as high as 38.5%. High anti-HBc prevalences of 58.6%[10] and 72.4%[13] were reported, assuming that horizontal infection might have occurred more frequently in adults or after childhood. Additionally, the significant differences in the HBsAg positive rate between residences suggests that HBsAg positive rates differ according to areas with different lifestyles in Cambodia.

From multivariate analysis, we found that men were significantly related to positivity for HBV infection (adjusted OR, 2.0), while there were no significant sex difference in positivity for HBsAg. This difference may suggest that men have higher risk for anti-HBc positivity; that means men have a higher risk for horizontal HBV transmission than women.

In this study, from logistic regression analysis, the older age groups had a higher risk for HBV infection. However, the HBsAg positive rate was not significantly different across the age groups. This discrepancy also suggested ongoing horizontal transmission of HBV among Cambodian adults; HBV infection rate is accumulated and grows higher with older age. Another reason why the older age group showed the higher positive rate of HBV infection would be a cohort effect.

Multivariate analysis particularly showed that history of operation is a potential risk factor for HBV infection in Cambodia (adjusted OR, 1.9). This result strongly supports HBV horizontal transmission. Therefore, even though the medical field in Cambodia now is restricted by poor hygiene due to its low economic condition, it is necessary to enhance the safety of medical treatment and launch a HB vaccination program for adults to prevent HBV infection.

Moreover, all HBV DNA positive samples belonged to genotype C, which was also reportedly dominant in Cambodia.[9,14] Our result is similar to those in previous studies. In Japan, where genotype C is predominant as in Cambodia, a study among HBV carriers in the birth cohort during 1950–1985 estimated that horizontal transmission was approximately sesquialterally as frequent as vertical transmission.[15]

On the other hand, our study showed that HCV RNA positive rate was 2.3% ($n = 483$) and anti-HCV positive rate was 5.8% in the adults among the general population in Siem Reap Province, Cambodia. Although anti-HCV rates were variously reported in the range of 2.3–14.7%,[10,11,13,16] our results clarified that the prevalence of HCV infection in Cambodia was high.

Table 2 Univariate and multivariate analyses of risk factors for HBsAg seropositivity, HBV infection among the general population in Siem Reap, Cambodia

	n	HBsAg						HBV infection†					
		Univariate analysis‡			Multivariate analysis§			Univariate analysis‡			Multivariate analysis¶		
		OR	(95% CI)	*P*	AOR	(95% CI)	*P*	OR	(95% CI)	*P*	AOR	(95% CI)	*P*
Sex													
Male	194	1.8	(0.8–4.3)	0.1591	2.3	(0.9–6.2)	0.0786	1.6	(1.1–2.3)	0.0191*	2.0	(1.3–3.1)	0.0029*
Female	289	1			1			1			1		
Age group (years)													
18–29	131	1			1			1			1		
30–39	121	2.6	(0.8–8.5)	0.1159	1.8	(0.5–7.0)	0.3532	2.0	(1.1–3.4)	0.0137*	1.9	(1.1–3.4)	0.0270*
40–49	103	2.0	(0.5–7.2)	0.2980	1.1	(0.3–4.7)	0.8958	2.6	(1.5–4.5)	0.0007*	2.5	(1.4–4.6)	0.0030*
50–59	66	0.5	(0.05–4.5)	0.5170	0.3	(0.02–2.4)	0.3379	3.3	(1.8–6.1)	0.0001*	3.4	(1.7–6.8)	0.0006*
60–89	62	1.1	(0.2–5.9)	0.9486	0.7	(0.09–3.9)	0.7013	2.2	(1.2–4.2)	0.0132*	2.3	(1.2–4.7)	0.0169*
Residence													
KC	186	1			1			1			1		
CV	249	7.2	(1.6–31.3)	0.0024*	7.4	(2.1–47.4)	0.0085*	2.6	(1.7–4.0)	<0.0001*	2.6	(1.7–4.1)	<0.0001*
RV	48	4.0	(0.6–29.2)	0.1407	4.0	(0.4–36.3)	0.1873	2.5	(1.3–4.8)	0.0053*	2.4	(1.2–5.1)	0.0172*
Occupation													
Farmer	266	0.6	(0.2–1.3)	0.1857	–			0.9	(0.6–1.3)	0.5989	–		
Others	202	1						1					
Healthy													
Yes	180	0.5	(0.2–1.3)	0.1448	0.3	(0.1–0.9)	0.0513	0.8	(0.6–1.2)	0.3464	0.8	(0.5–1.2)	0.2890
No	301	1			1			1			1		
Current periodic treatment													
Yes	174	0.8	(0.3–2.0)	0.6367	–			1.0	(0.6–1.4)	0.8054	0.8	(0.5–1.2)	0.2660
No	302	1						1			1		
History of disease or major injury													
Yes	199	1.2	(0.5–2.8)	0.6782	–			1.4	(0.9–2.0)	0.1120	–		
No	284	1						1					
History of injection or infusion													
Yes	369	0.8	(0.3–2.1)	0.6367	–			1.1	(0.7–1.6)	0.8220	–		
No	111	1						1					
History of operation													
Yes	46	0.9	(0.2–4.1)	0.9309	–			2.2	(1.2–4.1)	0.0100*	1.9	(1.0–3.7)	0.0485*
No	432	1						1			1		
History of blood transfusion													
Yes	5	0	–‡	0.6223	–			1.1	(0.2–6.6)	0.9257	–		
No	432	1						1					
Tattoo													
Yes	48	0.9	(0.2–3.9)	0.8456	–			1.2	(0.7–2.3)	0.4774	–		
No	354	1						1					
Holes for pierced earrings													
Yes	288	0.5	(0.2–1.4)	0.1863	–			0.6	(0.4–0.9)	0.0240*	–		
No	133	1						1					

†HBV infection including HBsAg positive and/or anti-HBc positive, ‡χ^2-test or Fisher's exact test.
‡No positives for HBsAg.
§Logistic regression analysis with a stepwise selection method: $R^2 = 0.1207$, model $P = 0.0058$*, $n = 481$.
¶Logistic regression analysis with a stepwise selection method: $R^2 = 0.0872$, model $P < 0.0001$*, $n = 470$.
*Statistically significant variables.
AOR, adjusted odds ratio; CI, confidence interval; CV, Chrey village; HBc, hepatitis B core; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; KC, Krabei Riel commune; OR, odds ratio; RV, Rohal village.

Table 3 Univariate and multivariate analyses of risk factors for HCV RNA and anti-HCV seropositivity among general population in Siem Reap, Cambodia

	n	HCV RNA						Anti-HCV					
		Univariate analysis†			Multivariate analysis‡			Univariate analysis†			Multivariate analysis§		
		OR	(95% CI)	*P*	AOR	(95% CI)	*P*	OR	(95% CI)	*P*	AOR	(95% CI)	*P*
Sex													
Male	194	1.2	(0.4–4.1)	0.7174	–			1.0	(0.4–2.1)	0.9220	–		
Female	289	1						1					
Age group (years)													
18–29	131	1			1			1			1		
30–39	121	1.1	(0.07–17.5)	0.9550	1.2	(0.05–30.2)	0.9103	1.5	(0.3–6.7)	0.6240	2.5	(0.3–51.5)	0.4340
40–49	103	3.9	(0.4–38.1)	0.2080	4.2	(0.5–88.8)	0.2365	2.6	(0.6–10.8)	0.1627	4.8	(0.8–93.7)	0.1558
50–59	66	0	–¶	0.4767	0	(0–15.4)	0.9906	2.8	(0.6–12.7)	0.1772	5.5	(0.8–111.0)	0.1369
60–89	62	13.9	(1.6–118.4)	0.0020*	15.0	(2.4–290.4)	0.0143*	9.2	(2.5–34.4)	0.0001*	16.8	(3.0–314.0)	0.0084*
Residence													
KC	186	1						1			1		
CV	249	2.0	(0.5–7.7)	0.2930	–			5.6	(1.7–19.1)	0.0021*	6.2	(2.0–28.6)	0.0057*
RV	48	0	–¶	0.3758	–			5.5	(1.2–25.7)	0.0148*	7.4	(0.3–83.0)	0.1258
Occupation													
Farmer	266	0.2	(0.03–0.8)	0.0088*	0.2	(0.02–0.7)	0.0265*	0.5	(0.2–1.0)	0.0531	–		
Others	202	1			1			1					
Healthy													
Yes	180	0.4	(0.1–1.7)	0.1822	–			0.4	(0.2–1.1)	0.0715	0.3	(0.08–1.1)	0.0967
No	301	1						1			1		
Current periodic treatment													
Yes	174	1.5	(0.4–4.9)	0.5352	–			1.5	(0.7–3.3)	0.2634	–		
No	302	1						1					
History of disease or major injury													
Yes	199	1.7	(0.5–5.8)	0.3630	–			1.1	(0.5–2.3)	0.8544	–		
No	284	1						1					
History of injection or infusion													
Yes	369	1.4	(0.3–6.4)	0.6940	–			2.6	(0.8–8.8)	0.1085	–		
No	111	1						1					
History of operation													
Yes	46	0.9	(0.1–7.5)	0.9517	–			1.1	(0.3–3.9)	0.8401	–		
No	432	1						1					
History of blood transfusion													
Yes	5	10.6	(1.1–103.1)	0.0121*	30.8	(1.0–575.3)	0.0231*	4.3	(0.5–39.5)	0.1667	12.0	(0.5–160.3)	0.0697
No	432	1			1			1			1		
Tattoo													
Yes	48	0	–¶	0.2156	–			0.3	(0.04–2.2)	0.2061	–		
No	354	1						1					
Holes for pierced earrings													
Yes	288	0.7	(0.2–2.5)	0.5627	–			1.1	(0.5–2.8)	0.7924	–		
No	133	1						1					

†χ^2-Test or Fisher's exact test.
‡Logistic regression analysis with a stepwise selection method: $R^2 = 02454$, model $P = 0.0003$*, $n = 426$.
§Logistic regression analysis with a stepwise selection method: $R^2 = 0.1879$, model $P < 0.0001$*, $n = 435$.
¶No positives for HCV RNA.
*Statistically significant variables.
AOR, adjusted odds ratio; CI, confidence interval; CV, Chrey village; HCV, hepatitis C virus; KC, Krabei Riel commune; OR, odds ratio; RV, Rohal village.