

<短 報>

高感度 HBs 抗原測定試薬 (BLEIA 法) を用いた検出性能に関する評価

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緒言：厚生労働省健康増進事業に基づく肝炎ウイルス検診等実施要領¹⁾では、HBs 抗原検査は凝集法等を用いて、HBs 抗原の検出を行い陽性又は陰性を判定することとなっている。無症状の住民を対象とした集団検診においては高感度の HBs 抗原測定は必須とはいえず²⁾、凝集法等による HBs 抗原陽性と判定された場合に医療機関受診を勧奨することにより所期の目的を達する。一方、HBV 再活性化の予防診断や慢性 B 型肝炎のモニタリングとしては HBV DNA 量が用いられているが、HBV DNA の感度に相当し、かつ動態を反映しうる HBs 抗原測定が可能であれば、安価で簡便なモニタリングとなり得る。

今回、HBV 感染初期の動態を経時的に観察したチンパンジーの血漿検体³⁾を用いて HBV DNA 量と、microparticle enzyme immunoassay (MEIA 法；以下 MEIA) による HBs 抗原及び生物発光酵素免疫測定法 Bioluminescent enzyme immunoassay (BLEIA 法；以下 BLEIA) による HBs 抗原について感度及び経時的動態の比較検討を行った。

対象および方法：1. 対象：

HBV 最小感染価を明らかにする目的で実施したチンパンジーを用いた感染実験³⁾で採取後、凍結保存したチンパンジー血漿検体を対象とした。この実験では、ヒト HBV 陽性血漿 Genotype A をチンパンジー No. 246, 279 および 280 の 3 頭にそれぞれ 6.9×10^4 copies, 10 copies, 10 copies, また、ヒト HBV 陽性血漿 Genotype C を No. 272, 269 および 285 の 3 頭にそれぞれ 2.7×10^6 copies, 10 copies, 10 copies 接種した。接種直後から 2 日～1 週間の間隔で採取した最大 441 日目までの HBV 感染初期の血漿検体計 281 例を対象とした。

2. 方法：

HBV DNA の定量は TaqMan[®]PCR(カットオフ値 2.1 log copies/ml, ロッシュ・ダイアクノスティックス(株)), HBs 抗原は BLEIA 法 (BLEIA[®]; カットオフ値 0.005 IU/ml, 栄研化学(株))および MEIA 法 (AXSYM[®], カットオフ値 1 S/CO, アボットジャパン(株))で測定した。また、HBc 抗体と HBs 抗体の測定は MEIA 法 (AXSYM[®], アボットジャパン(株))を用いた。各試薬の判定基準を合わせるため、カットオフ値を 1 としたカットオフインデックス値 (シグナル比=S/CO) の推移で比較した。

結果：HBV 感染初期における血漿中の HBV DNA 量と HBs 抗原測定結果を比較した (Fig. 1)。HBV genotype A を接種した No. 246・279・280 の 3 頭では、HBV DNA が検出感度 10^2 copies/ml 以上となったのは接種後 17, 55, 76 日であり、HBs 抗原が cut off 以上となったのは、MEIA では、35, 69, 97 日、BLEIA では、それぞれ 23, 57, 76 日であった。一方、genotype C を接種した No. 272・269・285 の 3 頭では、HBV DNA が検出感度 10^2 copies/ml 以上となったのは、それぞれ 6, 35, 43 日であり、HBs 抗原が cut off 以上となったのは、MEIA では、22, 50, 64 日、BLEIA では、それぞれ 13, 41, 50 日であった。HBV DNA 及び HBs 抗原のいずれも genotype C に感染した個体が、genotype A に感染した個体より早期に検出された。HBV genotype A あるいは genotype C に感染したチンパンジーの感染初期の動態では、BLEIA による HBs 抗原は、HBV DNA より遅く検出されたが、MEIA による HBs 抗原より早期に検出可能であった。

HBV Genotype A に感染した No. 246 および HBV Genotype C に感染した No. 272 の経過をみると、感染初期ではいずれも HBV DNA 量と HBs 抗原測定値は直線的に増加し、HBc 抗体の出現と同時に増加が止まった。HBV DNA 量の動態と BLEIA による HBs 抗原測定値の動態は非常に近似しており、HBc 抗体が出現するまでの 93 検体を対象に、HBV DNA 量と BLEIA

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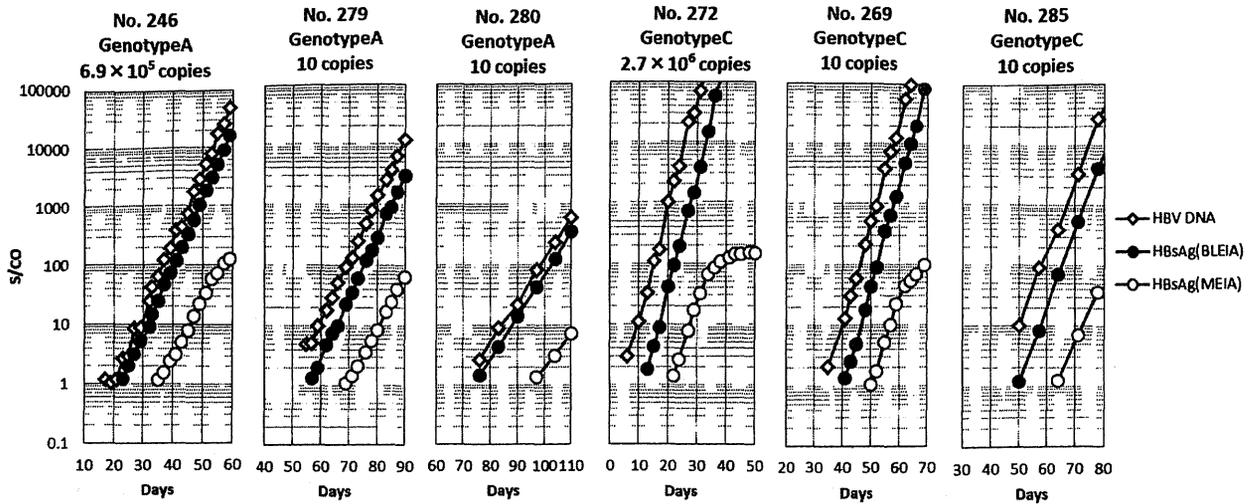


Fig. 1 Comparison of the cutoff index value in the detection early stage of HBV infection.

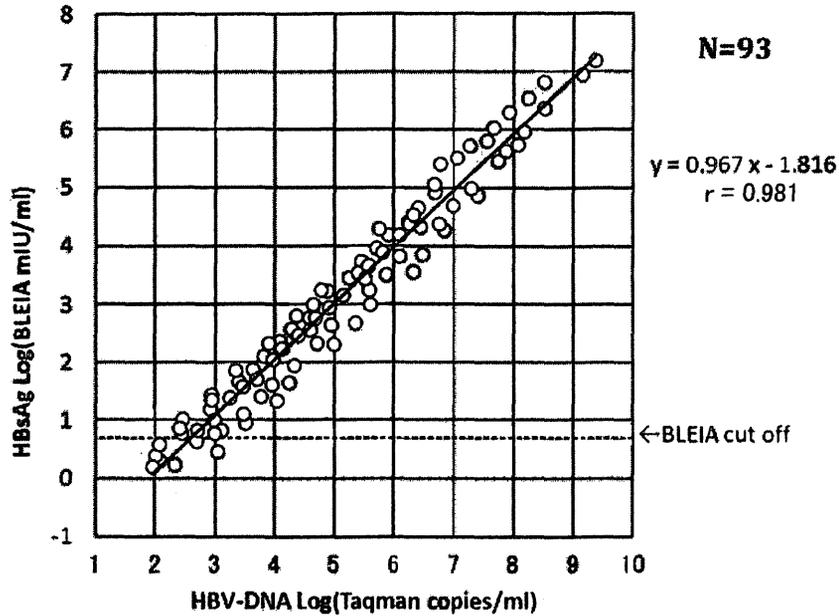


Fig. 2 Correlation between HBsAg (BLEIA) and HBV DNA

によるHBs抗原測定値 (Log (mIU/mL))を Fig. 2 に示した。 両測定値の相関性が高く(回帰式 $r=0.981$, $y=0.967x-1.816$), BLEIA による検出感度 0.005 IU/ml は HBV DNA 400 copies/ml に相当すると推定された。

考察：B型肝炎ウイルス (HBV) はヒト以外ではチンパンジーに感染することが知られており、ヒトの代

替動物として1970年代よりチンパンジーを用いた感染実験が行われ⁴⁾、ヒトのHBV感染病態を明らかにしている。独立行政法人理化学研究所が2002年1月に発表したチンパンジーとヒトのゲノムの相同性は98.77%と高い値を示している。これまで我々は2000~2006年度にチンパンジーを用いた感染実験を行い、チンパンジー血漿は生化学検査値には差異はないこと、遠心分離後の血漿回収率が低い傾向にあること等を認めて

いる。チンパンジーにおける血中の HBV 動態はヒト検体と同一であると判断して、チンパンジー血漿を用いた評価を行った。

本研究において HBc 抗体が出現するまでの HBV 感染早期の HBV DNA と HBs 抗原の動態をみると、BLEIA の検出感度 0.005 IU/ml は HBV DNA の 400 copies/ml 相当と推定され、ヒト検体を用いた Minekawa らの報告⁵⁾による BLEIA と同等の感度を認めた。TaqMan[®]PCR による HBV DNA の検出感度が 100 copies/ml であることから、BLEIA は高感度であると考えられた。

HBV の感染早期を経時的に追跡して採取したチンパンジー血漿を用いて高感度 HBs 抗原を測定した結果、HBV DNA に比べて検出感度は劣るが、従来の免疫測定試薬に比べ、HBV の早期検出が可能となり、かつ HBV DNA と良好な経時的動態の相関性を示した。HBs 抗原の消失などを含む慢性 B 型肝炎の長期病態の経過をみる指標として本法による HBs 抗原の測定は有用であると考えられた。

索引用語：

HBs 抗原,
Bioluminescent enzyme immunoassay (BLEIA),
チンパンジー

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本論文内容に関連する著者の利益相反：なし

英文要旨

Evaluation of the sensitivity of bioluminescent enzyme immunoassay for the detection of hepatitis B surface antigen

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The aim of this study was to evaluate the sensitivity of the bioluminescence enzyme immunoassay for the detection of hepatitis B surface antigen (BLEIA[®] EIKEN HBsAg) and to compare the sensitivity with microparticle enzyme immunoassay (MEIA). Samples were collected during the early infection phase from six chimpanzees infected with hepatitis B virus (HBV) genotype A or genotype C. In the early infection phase, there was a direct correlation between HBV DNA and HBsAg until the appearance of anti-HBc and anti-HBs. The cut off value of BLEIA, 0.005 IU/mL, was estimated to correspond to 400 copies/mL. BLEIA detected HBsAg earlier than other methods. Therefore, the measurement of HBsAg by BLEIA is suggested to be useful for the investigation of HBV infection.

Key words: hepatitis B surface antigen,
bioluminescent enzyme immunoassay,
chimpanzee

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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

	HBsAg positive			Anti-HBs positive			Anti-HBc positive			HCV RNA positive			Anti-HCV positive			
	n	(%)	(95% CI)	n	(%)	(95% CI)	n	(%)	(95% CI)	n	(%)	(95% CI)	n	(%)	(95% CI)	
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)	87	(44.9)	(37.8–51.8)	5	(2.6)	(0.35–4.8)	11	(5.7)	(2.4–8.9)
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)
Age group (years)																
18–29	131	4	(3.1)	(0.11–6.0)	26	(19.9)	(13.0–27.0)	32	(24.4)	(17.1–31.8)	1	(0.76)	(0–2.3)	3	(2.3)	(0–4.9)
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)
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)	47	(25.3)	(19.0–31.5)	3	(1.6)	(0–3.4)	3	(1.6)	(0–3.4)
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).

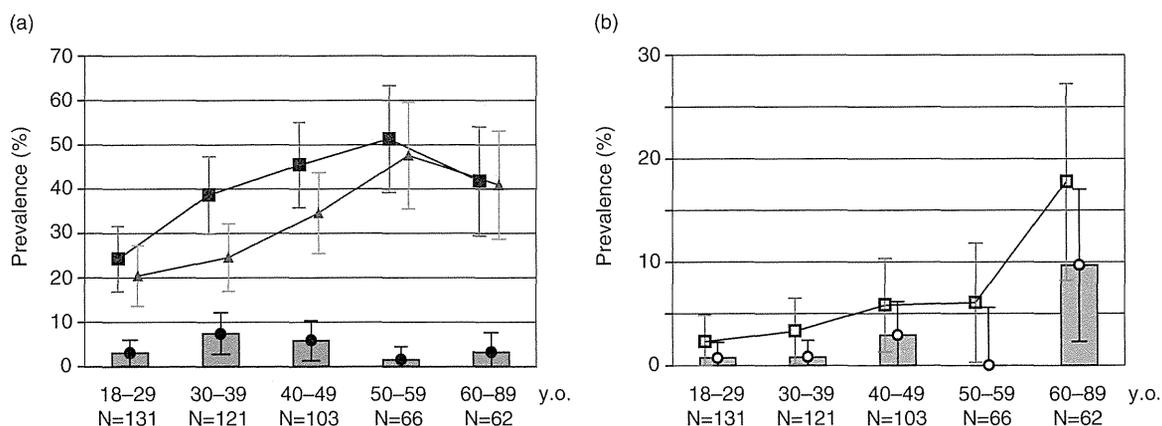


Figure 1 Age-specific prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection markers among the general population in Siem Reap, Cambodia. (a) The prevalence of HBV infection markers including hepatitis B surface antigen (HBsAg), anti-hepatitis B core (anti-HBc) and hepatitis B surface 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. ■, HBsAg; ■, 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: $R^2 = 0.2454$, 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.

In this study, the most frequent HCV RNA was genotype 6 (54.5%), followed by genotype 1b (27.3%). Genotypes 6 and 1 were also reported in Cambodian persons.¹⁶ Furthermore, viral RNA was detected in 39.3% of anti-HCV positive samples. In Japan, where genotype 1b is predominant,⁶ 70% of the individuals with anti-HCV are assumed to have an ongoing infection with HCV RNA.^{17,18} The lower proportion of HCV RNA among anti-HCV positive persons in Cambodia compared to that in Japan was possibly caused by the difference of genotype. It is known that response to interferon-based therapy varies according to HCV genotypes. Compared to other HCV genotypes, the response rate to the therapy in genotype 1 is lower,^{19,20} and that in genotype 6 may be at an intermediate level.^{19,20} Another possibility is host-oriented factors such as their own immunity or food intake in Cambodian people.

Additionally, from our multivariate analysis, participants aged of 60 years or older had high risk for anti-HCV positivity (adjusted OR, 16.8) and HCV RNA positivity (adjusted OR, 15.0). High risk of HCV infection may be attributed to unsafe blood transfusion or other insufficiently sterilized medical manipulations in the past. Other possibilities may be cohort effect or age effect.

Furthermore, our multivariate analysis found that the history of blood transfusion is a potential risk factor for HCV RNA positivity in Cambodia (adjusted OR, 30.8). Although all Cambodian blood donors now are requested to be screened for HIV, HBV, HCV and syphilis according to the Ministry of Health, it is a necessary to perform the screening procedure more rigorously to avoid infections from contaminated blood.

In conclusion, our results of HBV and HCV seroprevalence, genotypic distribution and multivariate analysis for risk factors supposed that horizontal HBV transmission may be frequent in Cambodia. Particularly, operation and blood transfusion were identified as the risk factor for HBV and HCV infection, respectively. Therefore, for reducing HBV and HCV infections, it is necessary to launch a HB vaccination program for adults and to improve blood safety and sterilization in hospital in Cambodia.

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Original Article

High prevalences of hepatitis B and C virus infections among adults living in Binh Thuan province, Vietnam

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Aim: Vietnam is one of the countries with the highest mortality from liver cancer, which is mostly attributed to hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. For planning preventive strategies against these infections, we investigated prevalences of HBV and HCV infections among adults living in Binh Thuan, Vietnam.

Methods: Our study consisted of a serological survey for HBV and HCV infections and a questionnaire survey on their risk factors. The sample size was calculated based on anticipated rate of hepatitis B surface antigen (HBsAg). Subjects were randomly sampled using a multistage method. Confirmation and family-tree surveys were conducted to examine persistent HBV infection and intrafamilial HBV transmission, respectively.

Results: A total of 509 adults, comprised of 230 men (45.2%) and 279 women (54.8%), were enrolled. Prevalences of HBsAg, hepatitis B surface antibody and hepatitis B core antibody

were 15.3%, 60.3% and 71.7%, respectively. Most HBV DNA positive sera were classified as genotype B (75.3%) and C (11.7%). Of HBsAg positive subjects, 96.7% were persistently infected and one acutely HBV infected person was identified. Family-tree surveys suggested that horizontal extrafamilial HBV transmission might have been frequent. Prevalences of anti-HCV and HCV RNA were 3.4% and 1.8%, respectively. HCV genotype 6a was prominent (55.6%).

Conclusion: In Binh Thuan, prevalences of HBV and HCV infections are high, HBV genotype B and HCV genotype 6a are predominant, and horizontal HBV transmission may still occur. Therefore, raising the coverage of a universal HBV vaccination program may be an effective liver cancer control in Vietnam.

Key words: general population, hepatitis B virus, hepatitis C virus, seroepidemiology, Vietnam

INTRODUCTION

HEPATITIS B VIRUS (HBV) and hepatitis C virus (HCV) infections have been considered the most important etiology of hepatocellular carcinoma (HCC), which is the third most common cause of cancer death among men globally.¹ Worldwide, nearly 2 billion people have been exposed to HBV² and

170 million people are chronically infected with HCV.³ In the Asia–Pacific region, HBV is the leading cause of chronic hepatitis which can evolve into liver cirrhosis and HCC.⁴

Liver cancer has been the most frequent cause of cancer death in Vietnam.⁵ According to Globocan 2012, Vietnam is one of the countries with the highest age-standardized mortality of liver cancer of 23.7 (per 100 000 population).⁵ Approximately 90% of patients with HCC had evidence of HBV⁶ and approximately one-seventh of them were related to HCV.⁶ Meanwhile, prevalences of chronic HBV and HCV infections in Vietnam were estimated to be approximately 12.0% and 2.0%, respectively.⁶ Particularly, chronic HBV infection was predicted to remain endemic in the next decade, despite the achievement of the universal infant HBV vaccination program.⁷ However, data on prevalences of

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HBV and HCV infections among the general population in Vietnam are limited. There have been few population-based studies conducted in North Vietnam in the last 6 years, while nationwide researches have not been performed. Therefore, we investigated HBV and HCV infections for seroprevalence, genotypic distribution and risk factors among the adults living in Binh Thuan, a province located in South Vietnam. Our population-based study is hoped to provide updated and additional information for planning preventive strategies against HBV and HCV infections in Vietnam.

METHODS

Study design

WE CONDUCTED A cross-sectional study among adults aged 20 years or older in Binh Thuan province. Based on the anticipated hepatitis B surface antigen (HBsAg) rate of 20.0%, confidence level of 95% and precision of 5%, sample size was determined to be 510. Participants were included by multistage sampling. First, La Gi town was one of three cities/towns/districts randomly chosen from 10 cities/towns/districts of Binh Thuan province. Except La Gi town, in two of three areas it is difficult to conduct this survey. Second, three wards, including Binh Tan, Phuoc Hoi and Phuoc Loc, were randomly selected from nine wards/communes of La Gi town. Finally, participants were enrolled from the lists of residents aged 20 years or older in three selected wards by systematic random sampling.

Additionally, approximately 6 months after the initial survey, all initial participants in Binh Tan ward were recruited to the confirmation survey for differentiating acute from persistent HBV infection among HBsAg positive individuals, as well as detecting incident HBsAg positive cases and HCV infections among susceptible persons.

In addition, for investigating the intrafamilial transmission of HBV infection, all participants who were persistently infected HBV, named "index persons", identified in our confirmation survey as well as their family members were invited to participate in the family-tree survey.

Ethical issues

This study was approved by the Ethics Committee for Epidemiological Research of Hiroshima University in Japan and Binh Thuan Provincial Department of Health in Vietnam. Informed consents were provided by all participants.

Questionnaire survey

An unsigned questionnaire was used to obtain data on characteristics of participants such as age, sex, occupation and potential risk factors including current health status, family history of liver diseases, history of hospitalization, surgery, blood transfusion, dental procedure, injections, acupuncture, tattoos, skin-piercings, sharing of razors, manicure service and skin-cutting cures.

Serological testing

Approximately 10 mL of blood was drawn from each participant. After centrifugation, sera were kept at -30°C and transported to Hiroshima University. All sera were tested for HBsAg, hepatitis B surface antibody (HBsAb), hepatitis B core antibody (HBcAb) and HBV DNA. HBsAg was detected using a reversed passive hemagglutination assay (Mycell II HBsAg; Institute of Immunology, Tokyo, Japan). A chemiluminescence immunoassay was used to detect HBsAb (Architect Osabu; Abbott, Tokyo, Japan) and HBcAb (Architect Hbc II; Abbott). HBV DNA was detected by real-time polymerase chain reaction (PCR) (TaqMan Fast Universal PCR Master Mix (2X); Applied Biosystems, Foster City, CA, USA) and the cut-off point for HBV DNA was more than 10^2 copies. HBV genotyping was performed by enzyme immunoassay (Immunis HBV genotype EIA; Institute of Immunology), and some samples which could not be determined by enzyme immunoassay were identified by direct sequencing (BigDye Terminator v3.1 Cycle Sequencing Kit; Applied Biosystems). HBV exposure was defined as the seropositivity for HBsAg and/or HBcAb.

For HCV infection, HCV antibody (anti-HCV) and HCV RNA were detected by particle agglutination assay (Ortho HCV Ab PA test II; Ortho-Clinical Diagnostics, Tokyo, Japan) and real-time PCR (TaqMan Fast Virus 1-Step Master Mix; Applied Biosystems), respectively. HCV infection was defined as the seropositivity for anti-HCV.

Statistical analysis

Data were analyzed using JMP version 7.0.2 (SAS Institute, Cary, NC, USA). The proportions were estimated with 95% confidence intervals (95% CI). The χ^2 -test or Fisher's exact test were used as appropriate to compare the proportions between different groups. Risk factors for HBV and HCV infections were determined by calculating odds ratios (OR) and 95% CI using univariate and multivariate logistic regression analyses with the stepwise method in which factors were retained if they

Table 1 Demographic characteristics of participants ($n = 509$)

Characteristics	Total		Men		Women	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Age (years)						
20–29	130	25.5	65	12.8	65	12.8
30–39	106	20.8	53	10.4	53	10.4
40–49	126	24.8	53	10.4	73	14.3
≥ 50	147	28.9	59	11.6	88	17.3
Occupation						
Farmer	75	14.7	35	15.2	40	14.3
Fisherman	23	4.5	23	10.0	0	0
Health-care worker	19	3.7	4	1.7	15	5.4
Housewife	112	22.0	0	0	112	40.1
Officer	128	25.2	79	34.4	49	17.6
Trader	66	13.0	18	7.8	48	17.2
Worker	86	16.9	71	30.9	15	5.4
Total	509	100.0	230	45.2	279	54.8

reached the 0.25 level of significance. A *P*-value less than 0.05 was considered statistically significant.

RESULTS

Characteristics of participants

IN 2012, 509 participants, comprised of 230 men (45.2%) and 279 women (54.8%), were enrolled in the study (Table 1). Age distribution ranged 20–81 years old (average age, 40.8 ± 1.1 years).

Among 169 initial subjects in Binh Tan ward, 152 (89.9%) participated in the confirmation survey. There was no significant difference in sex and age between subjects participating in the initial survey and those in the confirmation survey.

For family-tree surveys, four among 29 participants who were identified to be persistently infected with HBV agreed to participate. In total, 26 participants including four index persons and their 22 family members (parents, spouse or siblings of index persons) were studied. Their age ranged 23–75 years old and the male-to-female ratio was 1:1.36.

Questionnaire survey

Overall, 20.0% of participants had a family history of liver disease. Most participants were exposed to injections (78.4%), while fewer participants had history of surgery (18.9%), blood transfusion (6.1%) and tattoos (6.7%) (Fig. 1).

Serological analyses

Prevalences of HBsAg, HBsAb and HbCAb among participants were 15.3% (95% CI, 12.2–18.5%), 60.3%

(95% CI, 56.0–64.6%) and 71.7% (95% CI, 67.8–75.6%), respectively. Based on different profiles of HBV markers, prevalence of HBsAg positivity was 15.3% (78/509), while prevalence of HBV exposure (positive for HBsAg and/or HbCAb) was 71.7% (365/509). Among

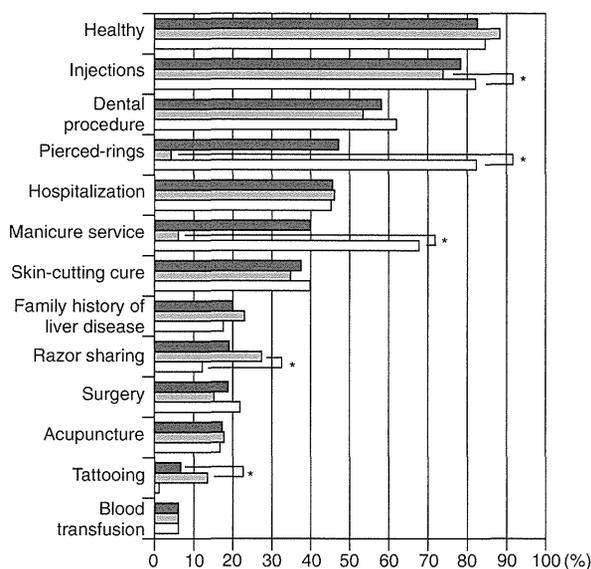


Figure 1 Potential risk factors among male and female participants in the sero-epidemiological study in Binh Thuan, Vietnam in 2012 ($n = 509$). Differences in percentages of "yes" answer between sexes were tested by χ^2 -test or Fisher's exact test as appropriate, and significant differences are indicated by asterisks. ■, Total ($n = 509$); ▒, male ($n = 230$); □, female ($n = 279$).

the 77 HBV DNA positive samples, 58 (75.3%) were classified as genotype B, nine (11.7%) as genotype C, four (5.2%) as genotype D and the remaining six (7.8%) as unidentifiable.

Generally, prevalences of these HBV markers in men and women were not significantly different (Fig. 2). Conversely, the rates of HBcAb positivity and HBsAb positivity tended to increase with age, while the HBsAg positive rate was inclined to decrease with age (Fig. 2). In univariate analysis, HBsAg seropositivity was related to age of 50 years or over (OR = 0.3; 95% CI, 0.1–0.6; $P < 0.001$) and family history of liver disease (OR = 3.1; 95% CI, 1.9–5.3; $P < 0.0001$), while HBV exposure was associated with age of 40–49 years (OR, 1.8; 95% CI = 1.0–3.0; $P < 0.05$) and age of 50 years or over (OR, 1.8; 95% CI = 1.1–3.1; $P < 0.05$) (Table 2). Multivariate analysis identified that HBsAg seropositivity was related to age of 50 years or over (adjusted OR = 0.3; 95% CI, 0.1–0.6, $P < 0.001$), fishermen (adjusted OR, 3.5; 95% CI = 1.1–10.1; $P < 0.05$) and family history of liver disease (adjusted OR = 3.0; 95% CI, 1.7–5.2; $P < 0.0001$), whereas HBV exposure was still associated with age of 40–49 years (adjusted OR = 1.8; 95% CI, 1.0–3.1; $P < 0.05$) and age of 50 years or over (adjusted OR = 1.8; 95% CI, 1.1–3.1; $P < 0.05$) (Table 2).

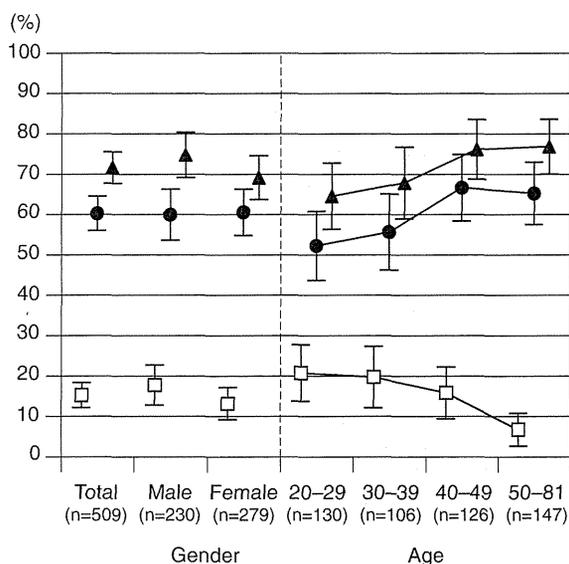


Figure 2 Prevalences of hepatitis B virus markers among adults living in Binh Thuan province, Vietnam. Sex- and age-specific prevalence of hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb) and hepatitis B core antibody (HBcAb) are shown with 95% confidence intervals. □, HBsAg; ●, HBsAb; ▲, HBcAb.

Notably, of the 31 HBsAg positive participants at the initial survey, 30 persons participated in the confirmation survey. Of the 30 participants, 29 (96.7%) remained positive for HBsAg, indicating persistent infection, whereas the remaining one became negative for HBsAg and positive for HBsAb, implying an acute infection at the initial survey. No HBsAg positive case was observed among subjects who were negative for all HBV markers at the initial survey, resulting in an incidence rate of 0 (95% CI, 0–29.5/100 person-years).

Additionally, in family-tree surveys, four index persons were positive for both HBsAg and HBcAb, whereas HBsAg was detected in eight of 22 family members. In total, 12 HBsAg positives among 26 persons in four families and the prevalence of HBsAg was 46.2% (95% CI, 27.0–65.3%). In family 1, the index person was the mother and only the oldest child was positive for HBcAb, while four younger children were negative for HBcAb (Fig. 3a). In families 2 and 3, the parents of the index persons were negative for HBsAg. However, HBsAg was detected in two index persons' older sisters in family 2, and four index persons' siblings in family 3 (Fig. 3b,c). The index person in family 4, the father, and his two sons were positive for HBsAg (Fig. 3d).

For HCV infection, 17 (3.3%; 95% CI, 1.8–4.9%) of the 509 participants were positive for anti-HCV, while HCV RNA was detected in nine (1.8%; 95% CI, 0.6–2.9%). Five (55.6%) of them were classified as genotype 6a. Each of the remaining four belonged to genotype 1b (11.1%), 2a (11.1%), 3a (11.1%) and 6e (11.1%), respectively. Differences in anti-HCV and HCV RNA rates between sexes were not significant (Fig. 4). Although anti-HCV and HCV RNA rates tended to increase with age (Fig. 4), only age of 50 years or over was related to seropositivity for anti-HCV in univariate analysis (OR = 8.4; 95% CI, 1.2–67.3; $P < 0.05$) and in multivariate analysis (adjusted OR = 6.4; 95% CI, 1.1–120.6; $P < 0.05$) (Table 3). In 150 participants who were initially negative for HCV infection, nobody was positive for anti-HCV or HCV RNA in the confirmation survey, resulting in a HCV incidence of 0 (95% CI, 0–4.9/100 person-years).

DISCUSSION

WE FOUND IN this survey that prevalence of HBsAg among the adults living in Binh Thuan, located in South Vietnam, was 15.3%. In North Vietnam, HBsAg rates of 18.8%,⁸ 19.0%⁹ and 8.8%¹⁰ were reported. Despite the discrepancies due to the

Table 2 Univariate and multivariate analysis of risk factors for HBV infection among adults living in Binh Thuan province, Vietnam (*n* = 509)

		<i>n</i>	%	HBsAg seropositivity						HBV exposure†					
				Univariate analysis‡			Multivariate analysis§			Univariate analysis‡			Multivariate analysis¶		
				OR	95% CI	<i>P</i>	AOR	95% CI	<i>P</i>	OR	95% CI	<i>P</i>	AOR	95% CI	<i>P</i>
Sex	Female	230	54.8	1.0			1.0			1.0					
	Male	279	45.2	1.4	0.9–2.3	0.1548	1.0	0.6–1.8	0.9050	1.3	0.9–1.9	0.1622	1.3	0.8–2.0	0.1575
Age group (years)	20–29	130	25.5	1.0			1.0			1.0			1.0		
	30–39	106	20.8	0.9	0.5–1.8	0.8557	0.8	0.4–1.6	0.5564	1.2	0.7–2.0	0.5932	1.1	0.6–1.9	0.9420
	40–49	126	24.8	0.7	0.4–1.4	0.3117	0.7	0.4–1.4	0.2893	1.8	1.0–3.0	0.0427	1.8	1.0–3.1	0.0416
	50 and over	147	28.9	0.3	0.1–0.6	0.0006	0.3	0.1–0.6	0.0009	1.8	1.1–3.1	0.0247	1.8	1.1–3.1	0.0258
Occupation	Worker	86	16.9	1.0			1.0			1.0			1.0		
	Farmer	75	14.7	1.0	0.4–2.3	0.9363	–			1.7	0.9–3.4	0.1321	–		
	Fisherman	23	4.5	2.5	0.8–7.1	0.0918	3.5	1.1–10.1	0.0223	1.9	0.7–5.7	0.2304	–		
	Health-care worker	19	3.7	1.1	0.3–4.1	0.9411	–			0.6	0.2–1.6	0.3081	0.4	0.2–1.3	0.1333
	Housewife	112	22.0	0.8	0.4–1.8	0.5949	–			1.5	0.8–2.7	0.2191	–		
	Officer	128	25.2	1.2	0.6–2.6	0.5844	–			1.4	0.8–2.6	0.2396	–		
	Trader	66	13.0	0.7	0.2–1.8	0.4149	–			1.5	0.8–3.1	0.2276	–		
Unhealthy status	No	439	86.3	1.0			–			1.0			–		
	Yes	70	13.7	1.0	0.5–2.1	0.9223	–			1.0	0.7–1.7	0.9552	–		
Family history of liver disease	No	407	80.0	1.0			1.0			1.0			1.0		
	Yes	102	20.0	3.1	1.9–5.3	<0.0001	3.0	1.7–5.2	<0.0001	1.5	0.9–2.4	0.1499	1.5	0.9–2.6	0.1182
Hospitalization	No	277	54.4	1.0			1.0			1.0			–		
	Yes	232	45.6	0.7	0.4–1.2	0.1702	0.7	0.4–1.2	0.1535	1.2	0.8–1.7	0.4727	–		
Surgery	No	413	81.1	1.0			1.0			1.0			–		
	Yes	96	18.9	0.6	0.3–1.3	0.2431	–			1.0	0.6–1.7	0.9681	–		
Blood transfusion	No	478	93.9	1.0			1.0			1.0			–		
	Yes	31	6.1	1.7	0.7–4.0	0.2471	2.4	0.9–6.2	0.0714	1.7	0.7–4.2	0.2543	–		
Dental procedure	No	213	41.9	1.0			1.0			1.0			–		
	Yes	296	58.1	0.7	0.4–1.1	0.1127	0.8	0.5–1.3	0.3084	0.9	0.6–1.3	0.5156	–		
Injections	No	110	21.6	1.0			1.0			1.0			–		
	Yes	399	78.4	0.9	0.5–1.6	0.7325	–			1.0	0.6–1.6	0.9771	–		
Acupuncture	No	421	82.7	1.0			1.0			1.0			–		
	Yes	88	17.3	0.9	0.4–1.6	0.6289	–			1.1	0.6–1.8	0.8157	–		
Tattoos	No	475	93.3	1.0			1.0			1.0			–		
	Yes	34	6.7	1.2	0.5–3.0	0.6971	–			1.3	0.6–3.0	0.5234	–		
Piercings	No	269	52.9	1.0			1.0			1.0			–		
	Yes	240	47.1	0.7	0.4–1.1	0.0948	–			0.8	0.5–1.2	0.2290	–		
Sharing of razors	No	412	80.9	1.0			1.0			1.0			–		
	Yes	97	19.1	1.3	0.7–2.4	0.3259	–			1.0	0.6–1.7	0.9118	–		
Manicure service	No	306	60.1	1.0			1.0			1.0			–		
	Yes	203	39.9	1.1	0.7–1.7	0.8226	–			0.8	0.6–1.2	0.3584	–		
Skin-cutting cures	No	318	62.5	1.0			1.0			1.0			–		
	Yes	191	37.5	0.9	0.5–1.4	0.5642	–			1.0	0.7–1.5	0.9943	–		

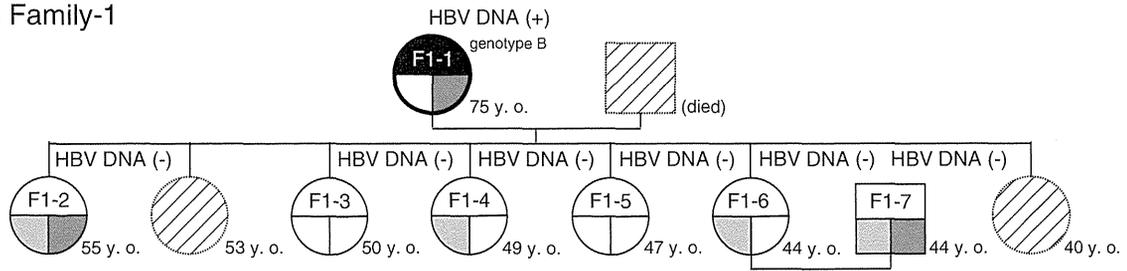
†Hepatitis B virus (HBV) exposure: seropositivity for hepatitis B surface antigen and/or hepatitis B core antibody.

‡ χ^2 -Test or Fisher's exact test.§Logistic regression analysis with the stepwise method: $R^2 = 0.0932$, model P -value < 0.0001 , $n = 509$.¶Logistic regression analysis with the stepwise method: $R^2 = 0.0236$, model P -value = 0.0262 , $n = 509$.

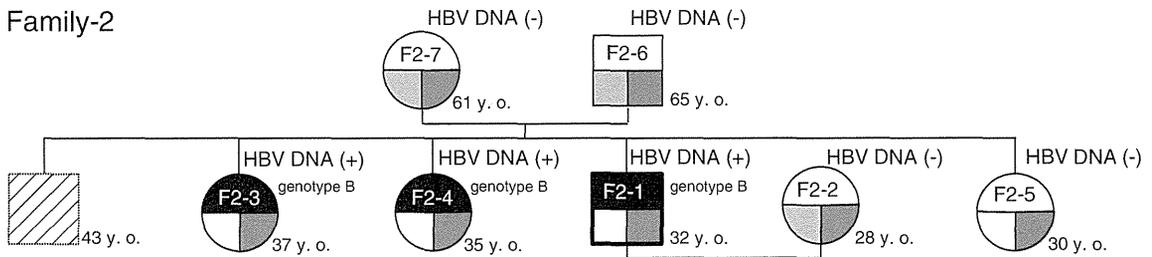
Bold text represents statistical significance.

AOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio.

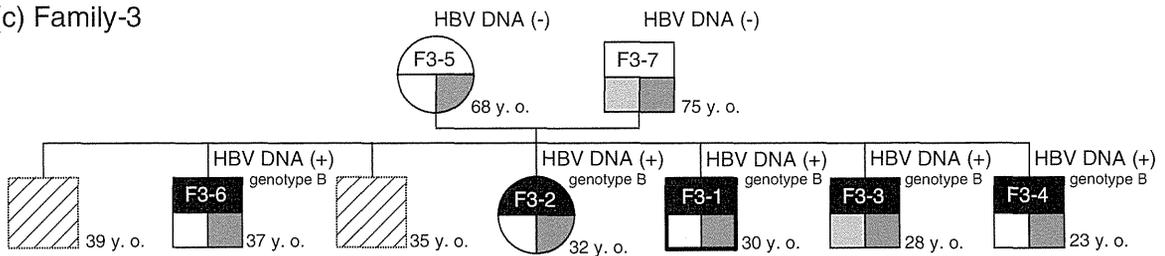
(a) Family-1



(b) Family-2



(c) Family-3



(d) Family-4

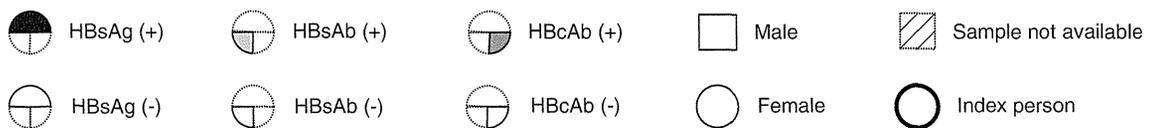
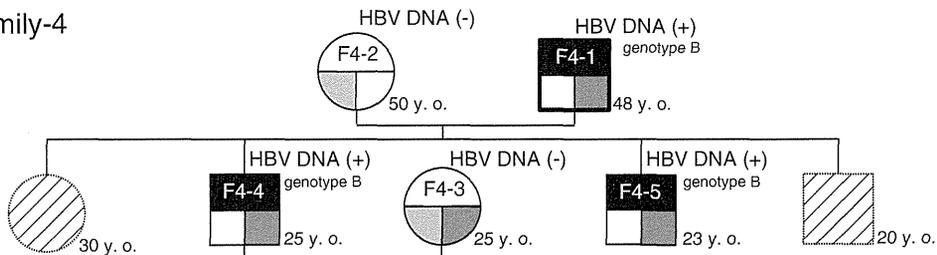


Figure 3 Trees of four families, including 26 subjects participating in the family-tree survey. Sex, age and results of hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), hepatitis B core antibody (HBcAb) and hepatitis B virus (HBV) DNA testings of each subject are shown. (a) Family (F)1; (b) F2; (c) F3; (d) F4. y. o., years old.

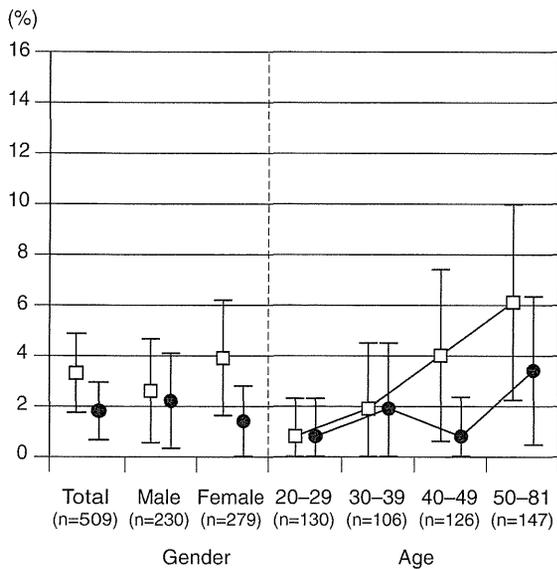


Figure 4 Prevalences of anti-hepatitis C virus (HCV) and HCV RNA among adults living in Binh Thuan province, Vietnam. Sex- and age-specific prevalence of anti-HCV and HCV RNA are shown with 95% confidence intervals. -□-, anti-HCV; -●-, HCV RNA.

differences in study areas, study periods of time, study methods and size, we can verify a high prevalence of HBsAg in Vietnam. According to conventional classification,² Vietnam remains an endemic country of HBV infection. Additionally, compared to other Southeast Asian nations^{4,11} and Japan,¹² Vietnam is one of the countries with the highest HBsAg seroprevalence. We observed that 71.7% of participants in our study were positive for HBcAb, the same high prevalence of 65.2–79.2%^{8,9} reported in North Vietnam. By comparing the prevalence of HBsAg and HBcAb in our study, we assumed that the horizontal infection may have occurred more frequently in adults or after childhood than by vertical transmission. Additionally, although the prevalence of HBsAb was high at 60.3% (307/509), only 62 (12.2%) participants were positive for HBsAb but negative for HBcAb. We supposed that their HBsAb seropositivity might not be related to the universal HBV vaccine program, because all participants had been born at least 10 years before the universal HBV vaccine program for infants was introduced in Binh Thuan in 2002. These 62 participants might have been vaccinated by themselves or might have been infected in the past with a low titer of HBcAb now.

Generally, genotypes B and C are most prevalent in Asian countries, while genotypes A and D are common

in Europe and the USA.¹³ Our study among the general population found that the most popular HBV genotype is B (75.3%), followed by C (11.7%). Comparable HBV genotype distribution of genotypes B and C were also observed in Vietnam.^{14,15} However, genotype C was reported to predominate over genotype B.^{16,17} These conflicts may be due to different study populations; genotype B was common among asymptomatic HBV infected cases, whereas genotype C was prominent in patients with liver cirrhosis and HCC.¹⁸

From the result of logistic regression analysis in our study, we did not find any sex difference in HBsAg positive risk and HBV exposure risk, the same result as in other surveys in Vietnam.^{9,10} However, a prevalence study in Japan reported that HBsAg positive rate among men was higher than that among women.¹² Moreover, we observed that persons aged 50 years or over had lower risk for HBsAg positivity than those aged 20–29 years. The same tendency was reviewed by Custer *et al.* in Southeast Asia.¹¹ The low HBsAg rates among older age groups might have been observed from two hypotheses: (i) the loss of HBsAg which was reported in the natural course of HBV carriers in Japan;¹⁹ and (ii) the cohort effect in Vietnam, such as the Vietnam War (1954–1975).

Hence, the evidences of age-dependent increase of HBcAb positive rate possibly imply ongoing horizontal transmission of HBV among Vietnamese adults. However, other reasons such as the differences in the historical background at different ages should be additionally investigated.

Notably, from our analysis we observed that family history of liver disease was a significant risk factor for HBsAg positivity (adjusted OR = 3.0). Therefore, family history of liver disease is considered an important predictor for HBV infection.

To our knowledge, this is the first study showing evidence of common HBV acquisition in early childhood in Vietnam through confirmation survey; most of the HBsAg positive adults were confirmed to be persistently infected. Additionally, we identified the case of acute HBV infection. At initial survey, this person was positive for HBsAg and HBcAb, and negative for HBsAb. Approximately 6 months later, at confirmation survey, the person became negative for HBsAg and positive for HBsAb and HBcAb. Hence, we suggest that ongoing HBV horizontal transmission in adulthood now occurs in Vietnam.

Furthermore, serological profiles in our family-tree surveys showed possibilities of different routes of HBV spread in Vietnam. Because taking blood samples at the same time for all members, particularly for children,

Table 3 Univariate and multivariate analysis of risk factors for HCV infection among adults living in Binh Thuan province, Vietnam ($n = 509$)

		n	%	Anti-HCV seropositivity						HCV RNA seropositivity					
				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	Female	230	54.8	1.0			1.0			1.0			1.0		
	Male	279	45.2	1.5	0.6–2.4	0.1548	1.2	0.4–3.6	0.6974	1.5	0.4–5.8	0.5283	1.6	0.4–6.8	0.4677
Age group (years)	20–29	130	25.5	1.0			1.0			1.0			1.0		
	30–39	106	20.8	2.5	0.2–27.7	0.4459	2.1	0.2–45.7	0.5537	2.5	0.2–27.7	0.4459	2.5	0.2–53.8	0.4606
	40–49	126	24.8	5.3	0.6–46.3	0.0908	4.4	0.7–85.8	0.1839	1.0	0.1–16.7	0.9823	1.1	0.0–27.4	0.9602
	50 and over	147	28.9	8.4	1.1–67.3	0.0171	6.4	1.1–120.6	0.0322	4.5	0.5–39.4	0.1331	4.8	0.8–92.4	0.1569
Occupation	Worker	86	16.9	1.0			1.0			1.0			1.0		
	Farmer	75	14.7	0.8	0.2–2.8	0.6665	–			0.6	0.1–3.2	0.5072	–		
	Fisherman	23	4.5	0	–	0.1925	–			0	–	0.2920	–		
	Health-care worker	19	3.7	0	–	0.2357	–			0	–	0.3378	–		
	Housewife	112	22.0	0.5	0.1–1.8	0.2781	–			0.4	0.1–2.1	0.2437	–		
	Officer	128	25.2	0.1	0.0–0.9	0.0125	0.2	0.0–1.2	0.1649	0	–	0.0138	–		
	Trader	66	13.0	0.4	0.1–2.1	0.2801	–			0.3	0.1–2.9	0.2826	–		
Unhealthy status	No	439	86.3	1.0			1.0			1.0			1.0		
	Yes	70	13.7	1.4	0.4–4.9	0.6353	–			0.8	0.1–6.3	0.8164	–		
Family history of liver disease	No	407	80.0	1.0			1.0			1.0			1.0		
	Yes	102	20.0	1.2	0.4–3.9	0.7146	–			1.1	0.2–5.6	0.8689	–		
Hospitalization	No	277	54.4	1.0			1.0			1.0			1.0		
	Yes	232	45.6	1.1	0.4–2.8	0.9009	–			1.5	0.4–5.7	0.5443	–		
Surgery	No	413	81.1	1.0			1.0			1.0			1.0		
	Yes	96	18.9	1.8	0.6–5.3	0.2580	–			2.2	0.5–8.9	0.2628	–		
Blood transfusion	No	478	93.9	1.0			1.0			1.0			1.0		
	Yes	31	6.1	1.0	0.1–7.5	0.9709	–			0	–	0.4408	–		
Dental procedure	No	213	41.9	1.0			1.0			1.0			1.0		
	Yes	296	58.1	1.3	0.5–3.7	0.5775	–			1.5	0.4–5.9	0.6014	–		
Injections	No	110	21.6	1.0			1.0			1.0			1.0		
	Yes	399	78.4	0.5	0.2–1.4	0.1633	–			0.5	0.1–2.2	0.3886	–		
Acupuncture	No	421	82.7	1.0			1.0			1.0			1.0		
	Yes	88	17.3	1.0	0.3–3.6	0.9683	–			0.6	0.1–4.8	0.6210	–		
Tattoos	No	475	93.3	1.0			1.0			1.0			1.0		
	Yes	34	6.7	0.9	0.1–6.8	0.8934	–			1.8	0.2–14.6	0.5911	–		
Piercings	No	269	52.9	1.0			1.0			1.0			1.0		
	Yes	240	47.1	2.1	0.8–5.8	0.1403	–			0.9	0.2–3.4	0.8696	–		
Sharing of razors	No	412	80.9	1.0			1.0			1.0			1.0		
	Yes	97	19.1	0.3	0–2.0	0.1595	0.2	0.0–1.3	0.1755	0.5	0.1–4.3	0.5403	–		
Manicure service	No	306	60.1	1.0			1.0			1.0			1.0		
	Yes	203	39.9	1.1	0.4–2.8	0.9117	–			0.8	0.2–3.0	0.6856	–		
Skin-cutting cures	No	318	62.5	1.0			1.0			1.0			1.0		
	Yes	191	37.5	0.9	0.3–2.5	0.8468	–			1.3	0.4–5.1	0.6653	–		

† χ^2 -Test or Fisher's exact test.‡Logistic regression analysis with the stepwise method: $R^2 = 0.0862$; model P -value = 0.0455, $n = 509$.§Logistic regression analysis with the stepwise method: $R^2 = 0.0457$, model P -value = 0.3883, $n = 509$.

Bold text represents statistical significance.

AOR, adjusted odds ratio; CI, confidence interval; HCV, hepatitis C virus; OR, odds ratio.

within a family is considered an unusual event in Vietnam, only four families of which a member was a health-care worker agreed to participate in family-tree survey. In family 1, there was no evidence of vertical HBV transmission as the mother was persistently HBV infected but none of her children were positive for HBsAg. In families 2 and 3, because the parents were negative for HBsAg, it was not obvious that children were infected from their parents. There was another possibility that the HBsAg positive children might have been horizontally infected from other persons who were persistently infected with HBV and had a close relationship with the children. In family 4, there was no evidence of vertical transmission because the mother was negative for HBsAg. These children might have been horizontally infected from their father or from other persons who were persistently infected with HBV and had a close relationship with them. We could not identify the transmission mode by using only genotyping results because all HBV DNA positive subjects in our family-tree surveys were infected with HBV genotype B. A recent study among HBV carriers in the birth cohort during 1950–1985 in Japan estimated that horizontal transmission was approximately sesquialterally as frequent as vertical transmission.²⁰ Therefore, it is possible that for the first time our family-tree survey has supposed that not only perinatal vertical transmission or intrafamilial horizontal spread but also horizontal infection of HBV from an extrafamilial source such as babysitter or kindergarten classmate in early childhood are important in Vietnam. Hence, preventive strategies for horizontal HBV transmission should be properly considered, along with the universal HBV vaccination for infants.

Regarding HCV infection, we revealed the anti-HCV seroprevalence of 3.3% (95% CI, 1.8–4.9%), higher than anti-HCV rate of 1.0% reported in North Vietnam.²¹ Particularly, our study is the first population-based investigation of HCV RNA prevalence in Vietnam, observing a HCV RNA positive rate of 1.8% (95% CI, 0.6–2.9%), much higher than the HCV carrier rate in Japan.²² Thus, in our study, 52.9% of anti-HCV positive individuals were seropositive for HCV RNA. This proportion was as high as 100.0% (3/3) in participants aged 20–39 years but declined to 42.9% (6/14) in participants aged 40 years or older, suggesting that the periods of infection were also different. Additionally, we identified that HCV genotype 6a was the most frequent (55.6%). In Vietnam, the predominance of HCV genotype 6a was also reported in blood donors²³ although HCV genotype 1b was more frequent among patients

with liver disease.²⁴ The discrepancy regarding HCV genotypic distribution may be explained by the fact that infection with HCV genotype 1b is more related with progressive liver diseases in comparison with other HCV genotypes.²⁵ Therefore, our results supposed that HCV genotype 6a was prominent among the general population.

From logistic regression analysis, we did not find any sex difference in the risk of anti-HCV seropositivity as well as in the risk of HCV RNA seropositivity. However, our multivariate analysis revealed that age of 50 years or over had a higher risk of anti-HCV seropositivity (adjusted OR = 6.4). High risk of anti-HCV seropositivity among the elderly may be attributed to unsafe blood transfusion and other insufficiently sterilized medical manipulations in the past.

With participants randomly sampled from the whole community, we provided sufficiently reliable results of seroprevalences of HBV and HCV infections. Overall, the high prevalence of HBcAb compared to that of HBsAg supposed that horizontal transmission was more frequent than expected. Another critical strength of this study is that results of both confirmation survey and family-tree surveys (in the former we could find one acutely HBV infected person among a small cohort during a short follow-up period, and in the latter all of children may not be vertically HBV infected) more strongly supported the importance of ongoing horizontal transmission.

Hence, to reduce the burden of HCC and other chronic liver diseases, it is crucial to raise the coverage of the universal infant HBV vaccination and launch a HBV vaccination program for adults in Vietnam. Other measures for preventing HBV and HCV parenteral transmission such as blood safety and sterilization in medical settings should be further improved.

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