Table 2 Symptoms, imaging findings and complications of patients with fulminant hepatitis (FH) and late-onset hepatic failure (LOHF)

	FH			LOHF ($n = 28$)
	Total ($n = 460$)	Acute type ($n = 227$)	Subacute type ($n = 233$)	
(a) Symptoms at diagnosis				
Fever†	13.0 (42/322)	17.5 (28/160)	8.6 (14/162)*	0 (0/23)*
Icterus	96.8 (427/441)	95.0 (208/219)	98.6 (219/222)*	96.4 (27/28)
Ascites	57.2 (237/414)	45.2 (88/204)	71.0 (149/210)**	81.5 (22/27)**
Convulsion	5.2 (22/422)	6.7 (14/210)	3.8 (8/212)	0 (0/27)
Tachycardia‡	36.7 (117/319)	39.5 (62/157)	34.0 (55/162)	47.8 (11/23)
Tachypnea§	34.5 (87/252)	39.1 (52/133)	29.4 (35/119)	31.6 (6/19)
Flapping tremor	79.0 (309/391)	75.8 (144/190)	82.1 (165/201)	80.8 (21/26)
Hepatic fetor	46.6 (146/313)	49.0 (73/149)	44.5 (73/164)	42.1 (8/19)
Pretibial edema	35.5 (127/358)	24.1 (42/174)	46.2 (85/184)**	75.0 (15/20)**,***
(b) Imaging findings				
Liver atrophy¶	58.8 (255/434)	45.6 (98/215)	71.7 (157/219)**	92.6 (25/27)**,***
(c) Complications				
Infection	34.8 (149/428)	32.9 (68/207)	36.7 (81/221)	51.9 (14/27)
Brain edema	18.5 (71/384)	24.1 (46/191)	13.0 (25/193)**	22.7 (5/22)
Gastrointestinal bleeding	13.2 (59/446)	11.0 (24/219)	15.4 (35/227)	20.0 (5/25)
Renal failure	38.9 (177/455)	40.9 (92/225)	37.0 (85/230)	39.3 (11/28)
DIC	34.6 (150/433)	35.7 (76/213)	33.6 (74/220)	53.8 (14/26)
Congestive heart failure	7.3 (31/427)	8.9 (19/214)	5.6 (12/213)	12.0 (3/25)

*$P < 0.05$, **$P < 0.01$ vs acute type, ***$P < 0.05$ vs subacute type.
†Temperature: >38°C or <36°C.
‡Heart rate: >90 beats/min.
§Respiratory rate: >20 breaths/min or $PaCO_2$: <32 Torr.
† ‡ § Cases between 2005 and 2009.
¶Liver atrophy detected by ultrasound and/or computed tomography imaging.
Data in parentheses indicate patient numbers.
DIC, disseminated intravascular coagulation.

reactivation, rituximab plus steroid combination chemotherapy was administrated to 35% of patients in inactive carriers and to 59% of those with transiently infected patients. HCV and HEV infection were less frequently found. In the survey, Epstein–Barr virus, herpes simplex virus and human herpes virus type-6 were found as other causes of viral hepatitis.

Autoimmune hepatitis was frequently observed in patients with the subacute type of FH and LOHF. Drug allergy-induced liver injury was observed in approximately 10–20% of patients irrespective of disease types. Anti-tuberculosis agents, non-steroidal anti-inflammatory drugs, anticancer agents, drugs for metabolic syndrome, and various herbal and natural remedies were the probable causative agents for this liver injury in the survey. Notably, the etiology was indeterminate in approximately 40% of patients with the subacute type of FH.

Therapies

For artificial liver support, plasma exchange and HDF were performed in most patients with FH (Table 4). Conventional HDF and continuous HDF (CHDF) were performed in 22.5% and 51.8% of patients with FH, respectively. A more powerful method, high-flow HDF (HF-HDF), high-flow CHDF (HF-CHDF) and on-line HDF were performed in 2.6%, 11.7% and 1.8% of the patients, respectively. The nucleoside analogs lamivudine and entecavir were used in approximately a quarter of patients with FH. Entecavir were used more frequently than lamivudine since 2007. Glucocorticosteroid, mainly as steroid pulse therapy, were administrated in more than 70% of patients with FH and LOHF. Anticoagulation therapy were performed in approximately 40–50% of patients with FH and LOHF. Glucagon/insulin, branched-chain amino acid-rich solution,

Table 3 Causes of fulminant hepatitis (FH) and late-onset hepatic failure (LOHF)

	FH			LOHF ($n = 28$)
	Total ($n = 460$)	Acute type ($n = 227$)	Subacute type ($n = 233$)	
Viral infection	46.1 (212)	62.6 (142)	30.0 (70)	32.1 (9)
HAV	3.0 (14)	5.7 (13)	0.4 (1)	0 (0)
HBV	40.2 (185)	54.2 (123)	26.6 (62)	32.1 (9)
(1) Transient infection	19.6 (90)	35.2 (80)	4.3 (10)	3.6 (1)
(2) Acute exacerbation in HBV carrier	14.1 (65)	7.9 (18)	20.2 (47)	25.0 (7)
(i) Inactive carrier, without drug exposure	7.4 (34)	6.2 (14)	8.6 (20)	3.6 (1)
(ii) Reactivation in inactive carrier†	3.3 (15)	1.8 (4)	4.7 (11)	17.9 (5)
(iii) Reactivation in transiently infected patient‡	3.5 (16)	0 (0)	6.9 (16)	3.6 (1)
(3) Indeterminate infection patterns	6.5 (30)	11.0 (25)	2.1 (5)	3.6 (1)
HCV	1.1 (5)	0.9 (2)	1.3 (3)	0 (0)
HEV	0.9 (4)	0.9 (2)	0.9 (2)	0 (0)
Other viruses	0.9 (4)	0.9 (2)	0.9 (2)	0 (0)
Autoimmune hepatitis	8.3 (38)	2.2 (5)	14.2 (33)	32.1 (9)
Drug allergy-induced liver injury	14.6 (67)	13.7 (31)	15.5 (36)	17.9 (5)
Indeterminate§	29.6 (136)	19.4 (44)	39.5 (92)	17.9 (5)
Unclassified¶	1.5 (7)	2.2 (5)	0.9 (2)	0 (0)

†Reactivation in inactive carrier by immunosuppressant and/or anticancer drugs.
‡Reactivation in transiently infected patients by immunosuppressant and/or anticancer drugs (de novo hepatitis).
§Indeterminate etiology despite sufficient examinations.
¶Unclassified due to insufficient examinations.
Data in parentheses indicate patient numbers.
HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus.

cyclosporin A and prostaglandin E_1 therapy were administrated less frequently compared with the previous survey.

Liver transplantation was performed in 23.5% and 17.9% of patients with FH and LOHF, respectively. Two patients received deceased-donor LT and 111 patients received living-donor LT. The frequency of LT was significantly greater in the subacute type than in the acute type of FH.

Prognosis

The prognosis of patients with FH and LOHF differed depending on the etiology (Table 5). Prognosis was good in patients with HAV infection. The prognosis was fair in patients with transient HBV infection. In contrast, the prognosis was poor in acute exacerbation in HBV carriers. The prognosis was extremely poor in patients with HBV reactivation, either from inactive carriers or transiently infected patients. Patients with the subacute type of FH and LOHF caused by autoimmune hepatitis, drug allergy-induced liver injury and indeterminate etiology also showed a poor prognosis.

The clinical features of the patients appeared to be associated with the prognosis. In the acute type of FH with no LT, the frequency of patients with SIRS (tachycardia or tachypnea) was greater in patients who died than in surviving patients ($P < 0.05$). Liver atrophy on ultrasound and/or computed tomography imaging was an important factor in predicting the prognosis of FH and LOHF with no LT. The frequencies were 25.0% and 64.5% in patients with the acute type ($P < 0.01$) and 55.6% and 78.1% in those with the subacute type of FH in surviving patients and those who died, respectively ($P < 0.05$).

Prognosis also appeared to be affected by complications. Any of the complications significantly decreased survival rate (data not shown). Furthermore, the number of these complications affected the prognosis. The survival rate of patients with the acute type of FH was greater than 80% in those with no complications, while it was less than 30% in those with two or more complications. The survival rate of patients with the subacute type of FH was decreased in proportion to the number of complications.

Table 4 Therapies for patients with fulminant hepatitis (FH) and late-onset hepatic failure (LOHF)

	FH			LOHF ($n = 28$)
	Total ($n = 460$)	Acute type ($n = 227$)	Subacute type ($n = 233$)	
Plasma exchange	90.9 (418/460)	92.5 (210/227)	89.3 (208/233)	71.4 (20/28)**,***
Hemodiafiltration	75.0 (342/456)	75.1 (169/225)	74.9 (173/231)	57.1 (16/28)
Glucocorticosteroids	72.4 (333/460)	68.3 (155/227)	76.4 (178/233)	89.3 (25/28)*
Glucagon/insulin	14.6 (67/459)	13.7 (31/227)	14.7 (34/232)	17.9 (5/28)
BCAA-rich solution	19.1 (87/456)	14.3 (32/223)	23.6 (55/233)*	39.3 (11/28)**
Prostaglandin E_1	7.0 (32/458)	6.7 (15/225)	7.3 (17/233)	3.6 (1/28)
Cyclosporin A	10.0 (46/460)	7.0 (16/227)	12.9 (30/233)*	10.7 (3/28)
Interferon	14.1 (65/460)	15.4 (35/227)	12.9 (30/233)	10.7 (3/28)
Nucleoside analog	38.9 (179/460)	50.9 (115/226)	27.5 (64/233)**	32.1 (9/28)
Lamivudine	25.5 (116/455)	40.0 (76/224)	30.4 (40/231)	12.5 (6/28)
Entecavir†	22.4 (70/312)	27.7 (41/148)	17.7 (29/164)	33.3 (5/15)
Anticoagulation therapy‡	47.2 (216/458)	43.2 (98/227)	51.1 (118/231)	39.3 (11/28)
Liver transplantation	23.5 (108/460)	15.9 (36/227)	30.9 (72/233)	17.9 (5/28)

*$P < 0.05$, **$P < 0.01$ vs acute type, ***$P < 0.05$ vs subacute type.
†Cases between 2006 and 2009.
‡Drugs such as antithrombin III concentrate and protease inhibitor compounds, gabexate mesylate and nafamostat mesilate.
Data in parentheses indicate patient numbers.
BCAA, branched-chain amino acid.

DISCUSSION

IN THIS SURVEY, 488 patients were enrolled over 6 years. In the previous 6-year survey, 697 patients (634 for FH and 64 for LOHF) were enrolled.[7] The incidence ratio of LOHF to FH was decreased from 9:1 to 16:1. In national epidemiology research, the annual incidence of FH was estimated at 1050 cases in 1996 and 429 cases in 2004.[11] Therefore, the incidence of FH and LOHF could be decreasing longitudinally. In this survey, the mean age of patients with FH and LOHF was older than that in the previous survey. More patients with complications received daily medication compared with the previous survey. Changes in demographic features of the patients may affect the etiology and prognosis of FH. A relationship between daily dose of oral medication and idiosyncratic drug-induced liver injury has been reported.[12] Additionally, older age is considered a poor prognostic factor in acute liver failure and may be considered a relative contraindication for LT.[13,14]

The current study showed that HBV still remains a major cause of FH and LOHF. Notably, almost half of acute exacerbations in HBV carriers occurred in patients with HBV reactivation owing to immunosuppressive or cytotoxic therapy. Approximately 80% of patients with transiently infected patients had received rituximab plus steroid combination therapy for non-Hodgkin's lymphoma. This combination therapy has been identified as a risk factor for HBV reactivation in HBsAg positive/negative patients with non-Hodgkin's lymphoma.[15,16] Our survey revealed that careful attention is necessary for transiently infected patients, as well as for inactive HBV carriers using intensive immunosuppressive agents.

The frequency of HAV infection in patients with FH was decreased compared with the previous survey. This result is compatible with no occurrence of outbreak of acute hepatitis A during this period. In Japan, zoonotic transmission from pigs, wild boar and deer, either foodborne or otherwise, is the cause of HEV infection.[17,18] In the currently studied survey, two-thirds of the patients were from endemic areas (Hokkaido Island and the northern part of mainland Honshu) in Japan.

The other principal finding in this survey was that the etiology was indeterminate in approximately 40% of patients with FH. One of the reasons for this result may be the failure of diagnosis for autoimmune hepatitis or drug-induced liver injury. Although the diagnosis of autoimmune hepatitis relies on the presence of serum autoantibodies, with higher IgG levels (>2 g/dL), acute-onset autoimmune hepatitis does not always show typical clinical features.[19–21] Additionally, the sensitivity of the drug-induced lymphocyte stimulation test for diagnosis is not completely reliable.

Table 5 Survival rates and etiology of patients with fulminant hepatitis (FH) and late-onset hepatic failure (LOHF) who did not have liver transplantation

	FH			LOHF ($n = 23$)
	Total ($n = 352$)	Acute type ($n = 191$)	Subacute type ($n = 161$)	
Viral infection	39.8 (70/176)	49.2 (58/118)	20.7 (12/58)**	14.3 (1/7)
HAV	57.1 (8/14)	61.5 (8/13)	0 (0/1)	–
HBV	36.2 (55/152)	46.1 (47/102)	16.0 (8/50)**	14.3 (1/7)
(1) Transient infection	52.6 (40/76)	54.4 (37/68)	37.5 (3/8)	–
(2) Acute exacerbation in HBV carrier	15.1 (8/53)	21.4 (3/14)	12.8 (5/39)	14.3 (1/7)
(i) Inactive carrier, without drug exposure	29.2 (7/24)	27.3 (3/11)	30.8 (4/13)	0 (0/1)
(ii) Reactivation in inactive carrier†	7.7 (1/13)	0 (0/3)	10.0 (1/10)	20.0 (1/5)
(iii) Reactivation in transiently infected patients‡	0 (0/16)	–	0 (0/16)	0 (0/1)
(3) Indeterminate infection patterns	30.4 (7/23)	35.0 (7/20)	0 (0/3)	–
HCV	50.0 (2/4)	100 (1/1)	33.3 (1/3)	–
HEV	75.0 (3/4)	100 (2/2)	50 (1/2)	–
Other viruses	100 (2/2)	–	100 (2/2)	–
Autoimmune hepatitis	32.4 (9/28)	40.0 (2/5)	30.4 (7/23)	12.5 (1/8)
Drug allergy-induced	32.8 (19/58)	43.3 (13/30)	21.4 (6/28)	0 (0/3)
Indeterminate§	37.6 (32/85)	54.5 (18/33)	26.9 (14/52)*	20.0 (1/5)
Unclassified¶	1.5 (7)	40.0 (2/5)	–	–

**$P < 0.01$ vs acute type.
†Reactivation in inactive carrier by immunosuppressant and/or anticancer drugs.
‡Reactivation in transiently infected patients by immunosuppressant and/or anticancer drugs (de novo hepatitis).
§Indeterminate etiology despite sufficient examinations.
¶Unclassified due to insufficient examinations.
Data in parentheses indicate patient numbers.
HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus.

Recently, powerful HDF using large buffer volumes (HF-HDF or HF-CHDF), or on-line HDF has been used. HF-HDF or HF-CHDF has a high recovery rate from a coma.[22–24] On-line HDF has an excellent recovery rate from a coma and is useful as a liver support system.[25] However, only 16% of patients with FH received these powerful HDF in the survey examined in the current study. The frequency of brain edema, gastrointestinal bleeding and congestive heart failure was decreased compared with that in the previous survey. Advances in artificial liver support and management may contribute to prevent these complications. Further evaluation is required to determine whether a new powerful support system can improve the prognosis of FH. The survival rate for FH patients with autoimmune hepatitis improved 17.1% in the previous survey to 32.4% in the 2004–2009 survey. Early commencement of corticosteroids may improve the prognosis. However, the efficacy of these drugs has not been evaluated statistically.

Recently, in patients with acute liver failure due to HBV, entecavir has been used more frequently than lamivudine because of its high potency and extremely low rates of drug resistance.[26] Entecavir beneficially affects the course of acute liver failure as lamivudine.[27,28] Despite the use of entecavir, the prognosis of HBV-infected patients, especially in HBV carriers, has not improved. In the case of HBV reactivation, it is difficult to prevent development of liver failure, even when nucleoside analogs are administrated after the onset of hepatitis. Because these agents require a certain amount of time to decrease HBV DNA in serum, they need to be administrated in the early phase of hepatitis. Guidelines for preventing HBV reactivation recommend the administration of nucleoside analogs before the start of immunosuppressive therapy in inactive carriers and at an early stage of HBV reactivation during or after immunosuppressive therapy in transiently infected patients.[29]

Despite new therapeutic approaches and intensive care, the prognosis of patients without LT with both types of FH and LOHF appeared similar to that in the previous survey. In contrast, the prognosis of patients receiving LT was good in the present survey. Yamashiki

et al. reported that the short-term and long-term outcomes of living-donor LT for acute liver failure were good, irrespective of the etiology and disease types.[30] In the current survey, the implementation rate of receiving LT was almost equivalent to that in the previous survey, irrespective of disease type. Notably, only two patients received deceased-donor LT in the current survey. Recently, patients with FH who received deceased-donor LT have been increasing since the new organ transplant bill passed in 2009. Hepatologists should realize that more donor action to increase deceased-donor LT is necessary to improve the prognosis of patients with FH or LOHF. Determining appropriate judgment to move forward to LT is the most important step. The indications for LT in cases of FH are determined according to the 1996 Guidelines of the Acute Liver Failure Study Group of Japan.[31] To improve the low sensitivity and specificity of assessment in patients with acute and subacute types,[32] new guidelines for using a scoring system have been established by the Intractable Hepato-Biliary Disease Study Group of Japan.[33] This novel scoring system showed sensitivity and specificity of 0.80 and 0.76, respectively, and greater than those in the previous guideline.[33] Recently, new prediction methods using data-mining analysis has been established.[34,35]

In conclusion, the demographic features and etiology of FH and LOHF have been gradually changing. HBV reactivation due to immunosuppressive therapy is a particular problem because of poor prognosis. The subacute types of FH and LOHF have a poor prognosis, irrespective of the etiology. Despite recent advances in therapeutic approaches, the implementation rate for LT and survival rates of patients without LT are similar to those in the previous survey.

ACKNOWLEDGMENT

THIS STUDY WAS performed with the support of the Ministry of Health, Labor and Welfare as an official project by the Intractable Hepato-Biliary Diseases Study Group of Japan.

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Hepatology Research

Hepatology Research 2013; 43: 1130–1138 doi: 10.1111/hepr.12075

Original Article

Impact of antibody to hepatitis B core antigen on the clinical course of hepatitis C virus carriers in a hyperendemic area in Japan: A community-based cohort study

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Aim: Subjects positive for antibody to hepatitis B core antigen (HBcAb) and negative for hepatitis B surface antigen (HBsAg) are considered to have occult hepatitis B virus (HBV) infection. The aim of this study was to determine the impact of occult HBV infection on aggravation of the clinical course in hepatitis C virus (HCV) carriers.

Methods: A prospective cohort study was performed in 400 subjects who were positive for anti-HCV antibody and negative for HBsAg. Among these subjects, 263 were HCV core antigen positive or HCV RNA positive (HCV carriers). We examined whether the presence of HBcAb affected the clinical course in these HCV carriers from 1996–2005.

Results: The HBcAb positive rates were 53.6% and 52.6% in HCV carriers and HCV RNA negative subjects, respectively. There were no differences in the incidence of hepatocellular carcinoma (HCC) and cumulative mortality associated with liver-related death between HCV carriers who were positive and negative for HBcAb. In multivariate analysis, age (≥65 years) and alanine aminotransferase level (≥31 IU/L) emerged as independent risk factors for HCC development and liver-related death, but the HBcAb status was not a risk factor. In addition, increased serum hepatic fibrosis markers (measured from 2001–2004) were not associated with HBcAb status.

Conclusion: In our cohort study, the presence of HBcAb had no impact on HCC development, liver-related death and hepatic fibrosis markers in HCV carriers. Thus, our results indicate that occult HBV infection has no impact on the clinical course in HCV carriers.

Key words: antibody to hepatitis B core antigen, hepatic fibrosis, hepatitis C virus, hepatocellular carcinoma, mortality, occult hepatitis B virus infection

INTRODUCTION

HEPATITIS B SURFACE antigen (HBsAg) is the most common serum marker for simple detection of hepatitis B virus (HBV) infection. It has previously been thought that HBsAg positive individuals were HBV carriers and that HBsAg negative individuals who were anti-hepatitis B core antibody (HBcAb) positive and/or anti-hepatitis B surface antibody (HBsAb) positive had a history of the infection, but were not HBV carriers. However, it has recently been established that HBV DNA is occasionally detected in blood and/or liver tissue in individuals who are HBcAb positive and/or HBsAb positive and HBsAg negative. Thus, HBsAg negative patients who are HBcAb and/or HBsAb positive are regarded to have occult HBV infection.[1,2] Such patients are suspected HBV carriers, even if they are negative for HBsAg,[3] and serum HBcAb is considered to be a serological marker of occult HBV infection.[4,5]

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Financial disclosure: The authors declare that they have nothing to disclose regarding funding or conflict of interest with respect to this study.
Received 19 December 2012; revision 14 January 2013; accepted 16 January 2013.

Hepatitis B core antibody is present in 9–25% of healthy individuals,[2,6,7] but in 46–78% of patients with hepatitis C virus (HCV)-related chronic liver disease.[3,5,8] The HBcAb positive rate in HCV carriers with hepatocellular carcinoma (HCC) has been reported to be higher than that in HCV carriers without HCC.[9] However, there is a debate concerning the effects of occult HBV infection on the pathology of diseases such as HCC in patients with HCV-related chronic liver disease. Previous studies of these effects have been performed in patients who visited hospitals for treatment of advanced chronic liver disease,[1,3,8] but not as cohort studies in community residents. Tanaka *et al.* found that a HBcAb positive status was a significant risk factor for development of HCC in a 12-year, but small scale ($n = 74$), prospective study in anti-hepatitis C antibody (HCVAb) positive community residents.[10] Thus, a large scale cohort study is needed to clarify whether occult HBV infection affects the natural course of HCV infection, and a long-term prospective study in community residents is desirable. To address this issue, we performed a community-based prospective cohort study in a HCV hyperendemic area of Japan to investigate the effects of occult HBV infection on prognosis, development of HCC and progression of hepatic fibrosis in community residents with HCV infection.

METHODS

Study population

THE SUBJECTS WERE residents in town C, a HCV hyperendemic area of Japan in which we performed a cohort study from 1993–2005.[11–13] Town C is a small town located in the mid-west of Miyazaki Prefecture in Kyushu, Japan. It has a HCVAb positive rate of 20.6%, which is higher than those in surrounding areas.[13] During a 10-year period beginning in 1995, 1125 anti-HCV seropositive residents of town C were enrolled in the previous study[11] and followed for mortality through 2005. Among the HCVAb positive subjects, 231 deaths occurred over a mean follow-up period of 8.2 years. Liver-related deaths occurred more frequently among HCV carriers, whereas the rates of other causes of death did not differ between HCV carriers and non-carriers.[11] The current study included 400 HCVAb positive and HBsAg negative subjects in whom HBcAb was measured before August 1996.

Follow up

This study was started in September 1996 and completed on 31 December 2005. Subjects with HCC shown by abdominal ultrasonography at the time of HBcAb measurement were excluded from the study. Abdominal ultrasonography was performed once a year when possible during follow up. During the course of this study, two subjects moved to other areas and follow up was stopped at this point. No other subjects were lost to follow up. Cause of death was based on information from death certificates. In 2001 and 2004, serum hyaluronic acid and type IV collagen 7S, which are hepatic fibrosis markers, were measured in 129 subjects.

Informed consent was obtained from subjects at the time of enrollment. The study was approved by the ethics committees of the University of Miyazaki, Faculty of Medicine, and the Kagoshima University Graduate School of Medical and Dental Sciences. In the study, HCV carriers were defined as individuals positive for HCV core antigen (HCVcAg) or HCV RNA. Subjects with occult HBV infection were defined as those positive for HBcAb, but negative for HBsAg.

Serum markers

Hepatitis C antibody was detected using a second-generation enzyme immunoassay kit (Immunocheck F-HCVAb; International Reagents, Kobe, Japan). Serum levels of HCVcAg were determined by a fluorescence enzyme immunoassay (Immunocheck F-HCVAg Core; International Reagents). For HCVAb positive subjects with HCVcAg levels of less than 8 pg/mL, HCV RNA was examined before 1996 by a qualitative reverse transcription polymerase chain reaction assay (Amplicore HCV; Roche Diagnostics, Tokyo, Japan). The serologically defined genotype (serotype) of HCV was determined using a serological genotyping assay kit (Immunocheck F-HCV Grouping; International Reagents). Detection of HBcAb was performed by radioimmunoassay (Dainabott, Tokyo, Japan). Hyaluronic acid and type IV collagen 7S were measured by a latex bead agglutination assay (LPIA-ACE HA; Mitsubishi Kagaku Iatron, Tokyo, Japan; normal, <50 ng/mL) and a radioimmunoassay (Type IV collagen 7S kit; Mitsubishi Kagaku Iatron; normal, <6.0 ng/mL), respectively.

Liver stiffness evaluated by elastography

Liver stiffness was evaluated by transient elastography (Fibroscan; Echosens, Paris, France) for 144 subjects in 2004. This is a rapid, non-invasive method that gives results that are correlated with the fibrotic stage of liver disease.[14] The measurements were made on the right lobe of the liver. The results are expressed in kilopascals (kPa).[15]

Statistical analysis

A χ^2-test, Fisher's exact test, Student's *t*-test or Mann–Whitney *U*-test was used to compare frequencies or means, as appropriate. Cumulative survival curves were constructed using the Kaplan–Meier method and analyzed by log-rank test. For multivariate analysis, logistic regression analysis or Cox proportional hazards models were used. Statistical analyses were performed using SPSS ver. 18 software (SPSS, Chicago, IL, USA), with $P < 0.05$ considered significant.

RESULTS

Characteristics of patients at enrollment

AMONG 400 SUBJECTS who were positive for HCVAb, 263 were HCV carriers (positive for HCVcAg or HCV RNA), including 141 HBcAb positive (53.6%) and 122 HBcAb negative (46.4%) subjects (Table 1). A total of 137 subjects were negative for both HCVcAg and HCV RNA, including 72 HBcAb positive (52.6%) and 65 HBcAb negative (47.4%) subjects. Among the HCV carriers, the HBcAb positive subjects were older than those who were HBcAb negative ($P = 0.02$) (Table 1). The HBcAb positive rates showed no sex differences in HCV carriers and HCV RNA negative subjects (Table 1, Fig. 1a), and these rates in subjects in their 50s to 70s were 40–60%, regardless of the status of HCV infection (Fig. 1b). There were also no significant differences in other characteristics between the two groups (Table 1).

Factors influencing the development of HCC

During the follow-up period, HCC developed in 35 of 263 HCV carriers, including 22 and 13 who were HBcAb positive and HBcAb negative, respectively, and in three of 137 HCV RNA negative subjects. Suspected liver cancer cases were identified in our liver disease screening programs using abdominal ultrasonography and the diagnosis of HCC was subsequently confirmed by primary physicians. For 36 cases of HCC, the diagnosis was determined on the basis of information collected via biopsy and/or imaging analysis using magnetic resonance imaging, computed tomography, angiography or ultrasonography. Two additional HCC cases were identified based on death certificates. Because the exact date of HCC onset was not available, the subjects were divided into two groups based on the presence or absence of development of HCC and

Table 1 Characteristics of patients at enrollment

Characteristics	HCV carrier† ($n = 263$)			HCV RNA(−) ($n = 137$)		
	HBcAb (+) $n = 141$ (53.6%)	HBcAb(−) $n = 122$ (46.4%)	*P*-value‡	HBcAb(+) $n = 72$ (52.6%)	HBcAb(−) $n = 65$ (47.4%)	*P*-value‡
Age (years)	65.1 ± 9.2	62.2 ± 9.8	0.02	65.6 ± 7.8	64.3 ± 8.3	0.36
Sex (male/female)	59/82	53/69	0.80	27/45	23/42	0.86
Alcohol, (never/occasionally/daily/unknown)	68/18/50/5	57/23/41/1	0.29	30/16/21/5	29/13/23/0	0.17
Blood transfusion (yes/no/unknown)	18/114/9	18/111/3	0.86	12/55/5	13/51/1	0.82
Previous interferon therapy (yes/no)	26/115	22/100	1.00	6/66	6/59	1.00
HCV serotype (I/II/indeterminate/not tested)	88/41/8/4	77/38/4/3	0.83			
HCV core antigen (pg/mL) (undetectable/<100/≥100/not tested)	14/45/80/2	12/45/65/0	0.53			
Platelet count ($\times 10^4/\mu L$)	18.0 ± 5.5 (114)	19.0 ± 4.5 (94)	0.16	21.3 ± 6.0	20.5 ± 5.4	0.47
Alanine aminotransferase (IU/L)	41.4 ± 34.3 (140)	45.3 ± 31.3	0.34	23.2 ± 17.5	24.7 ± 28.4	0.70

Data are shown as a number or mean ± standard deviation (number of subjects examined).
†HCV carriers were defined as subjects who were positive for HCV core protein or HCV RNA.
‡Based on χ^2-test, Fisher's exact test, or Student's *t*-test, as appropriate.
HBcAb, antibody to hepatitis B core antigen; HCV, hepatitis C virus.

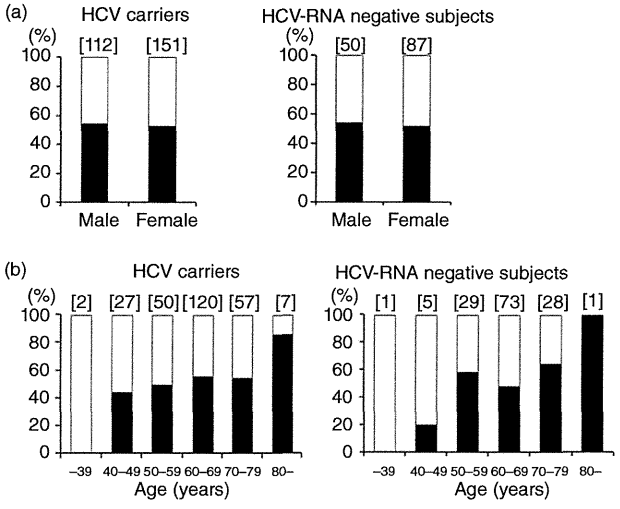

Figure 1 HBcAb positive rates in HCV carriers and HCV RNA negative subjects. The HBcAb positive rates by gender were similar in the two groups (a) and gradually increased with age in both groups (b). HBcAb, antibody to hepatitis B core antigen; HCV, hepatitis C virus. (Number of subjects examined.) ■, HbcAb (+); □, HbcAb (–).

differences in characteristics were investigated between these groups.

Univariate analysis indicated that age of 65 years or older, male sex, platelet count of less than 150 000/μL and alanine aminotransferase (ALT) of 31 IU/L or more were significantly related to development of HCC in HCV carriers. Multivariate analysis showed that age of 65 years or older and ALT of 31 IU/L or more were independent risk factors for HCC development (Table 2). In contrast, the presence of HBcAb was not a significant factor in these analyses.

Effect of occult HBV infection on death caused by liver diseases

During the follow-up period, death occurred in 67 of 263 HCV carriers and in 21 of 137 HCV RNA negative subjects. In these deaths, liver-related death such as HCC, liver cirrhosis and esophageal varices occurred in 33 HCV carriers, including 17 and 16 who were HBcAb positive and HBcAb negative, respectively, and in three HCV RNA negative subjects. There was no significant

Table 2 Univariate and multivariate analyses of variables associated with development of hepatocellular carcinoma

Variable	Status	Odds ratio	95% CI	*P*-value
Univariate analysis				
HBcAb	Negative	1		
	Positive	1.550	0.745–3.227	0.277
Age (years)	<65	1		
	≥65	2.372	1.089–5.164	0.029
Sex	Female	1		
	Male	2.258	1.092–4.669	0.028
HCV core antigen (pg/mL)	Undetectable	1		
	<100	2.026	0.427–9.618	0.374
	≥100	1.701	0.370–7.812	0.495
Alcohol	None	1		
	Occasionally	1.510	0.569–4.007	0.408
	Daily	1.008	0.440–2.312	0.984
Blood transfusion	No	1		
	Yes	0.583	0.168–2.025	0.589
Platelet count ($\times 10^4/\mu L$)	≥15	1		
	<15	3.220	1.324–7.829	0.011
ALT (IU/L)	≤30	1		
	≥31	4.427	1.766–11.1	0.001
Multivariate analysis†				
Age (years)	<65	1		
	≥65	4.308	1.198–15.495	0.025
ALT (IU/L)	≤30	1		
	≥31	5.803	1.625–20.727	0.007

†Based on logistic regression analysis.
ALT, alanine aminotransferase; CI, confidence interval; HBcAb, antibody to hepatitis B core antigen; HCV, hepatitis C virus.

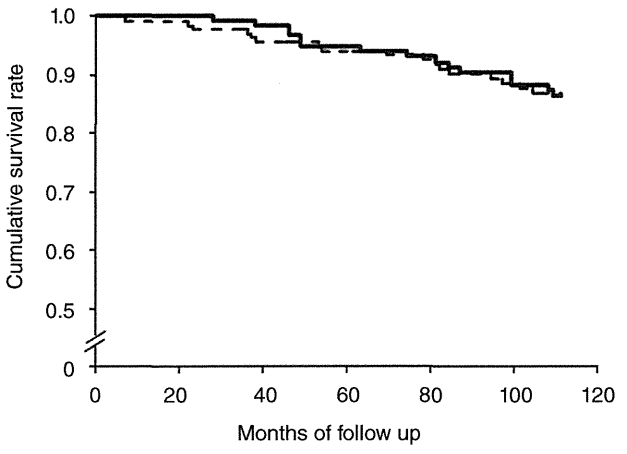

Figure 2 Cumulative survival rates associated with liver-related death in HBcAb positive and HBcAb negative HCV carriers, plotted using the Kaplan–Meier method. There was no significant difference between the two groups by log–rank test. HBcAb, antibody to hepatitis B core antigen; HCV, hepatitis C virus. —, HbcAb (+); ----, HbcAb (–).

difference in the cumulative survival rate calculated by the Kaplan–Meier method between HBcAb positive and HBcAb negative HCV carriers (Fig. 2).

Risk factors for death caused by liver diseases

Analysis using the Kaplan–Meier method identified age of 65 years or older, platelet count of less than 150 000/μL and ALT of 31 IU/L or more as significant risk factors for death caused by liver-related death. In multivariate Cox regression analysis, age of 65 years or older and ALT of 31 IU/L or more remained as significant independent risk factors, while HBcAb was not a risk factor (Table 3).

Effect of occult HBV infection on serum albumin levels and hepatic fibrosis markers in HCV carriers

Serum albumin levels measured in 2001 or 2004 were compared between HBcAb positive and HBcAb negative HCV carriers. The mean serum albumin levels in both

Table 3 Univariate and multivariate analyses of variables associated with liver-related death

Variable	Status	Hazard ratio	95% CI	*P*-value
Univariate analysis				
HBcAb	Negative	1		
	Positive	1.035	0.522–2.054	0.922
Age (years)	<65	1		
	≥65	2.157	1.026–4.534	0.043
Sex	Female	1		
	Male	0.982	0.488–1.975	0.959
HCV core antigen (pg/mL)	Undetectable	1		
	<100	0.588	0.152–2.275	0.442
	≥100	1.157	0.345–3.878	0.814
Alcohol	None	1		
	Occasionally	0.255	0.060–1.090	0.255
	Daily	0.519	0.23–1.172	0.115
Blood transfusion	No	1		
	Yes	0.619	0.188–2.039	0.430
Platelet ($\times 10^4/\mu L$)	≥15	1		
	<15	3.126	1.327–7.362	0.009
ALT (IU/L)	≤30	1		
	≥31	4.553	1.753–11.824	0.002
Multivariate analysis†				
Age (years)	<65	1		
	≥65	5.983	1.375–26.036	0.017
ALT (IU/L)	≤30	1		
	≥31	4.337	1.255–14.986	0.020

†Based on Cox proportional hazards models.
ALT, alanine aminotransferase; CI, confidence interval; HBcAb, antibody to hepatitis B core antigen; HCV, hepatitis C virus.

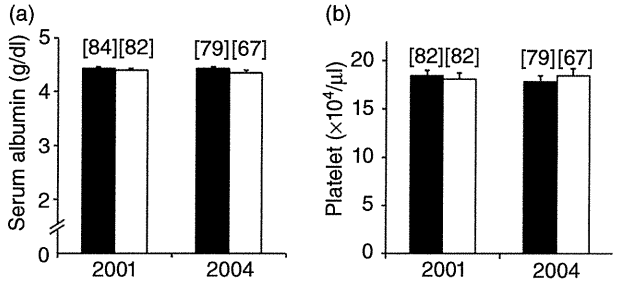

Figure 3 Serum albumin levels and platelet counts in HBcAb positive and HBcAb negative HCV carriers. Comparison of serum albumin levels (a) or platelet counts (b) obtained in 2001 or 2004. HBcAb, antibody to hepatitis B core antigen; HCV, hepatitis C virus. (Number of subjects examined.) ■, HbcAb (+); □, HbcAb (−).

groups were more than 4 g/dL in 2001 and 2004, with no significant difference between the groups (Fig. 3a). In addition, the serum albumin levels in 2004 were not significantly lower than those in 2001 in either group (62 subjects in each group) who underwent measurements in both years.

The effect of occult HBV infection on hepatic fibrosis was studied in HCV carriers using measurements made in 2001 or 2004. The platelet count, an index of hepatic fibrosis, did not differ between HBcAb positive and HBcAb negative HCV carriers in 2001 or 2004 (Fig. 3b). There was also no significant difference in platelet counts in 2001 and 2004 in subjects in each group who underwent measurements in both years (Fig. 4a). Further, there were no significant differences in the levels of hyaluronic acid and type IV collagen 7S, which are hepatic fibrosis markers, between HBcAb positive and HBcAb negative HCV carriers in 2001 or 2004. In contrast, the levels of these markers in 2004 were significantly greater than those in 2001 in both groups who underwent measurements in both years (Fig. 4b, c), but with no dependence on the presence or absence of HBcAb.

Hepatic fibrosis was evaluated in 144 HCV carriers using elastography in 2004. Mean values of 8.48 and 8.51 kPa were obtained in HBcAb positive ($n = 78$) and HBcAb negative ($n = 66$) HCV carriers, with no significant difference between the groups ($P = 0.67$).

DISCUSSION

OCCULT HBV INFECTION may influence development of HCC in patients with HCV infection, but this is an issue on which no consensus has been reached. The current study was performed as an approximately 10-year cohort study in community residents by defining occult HBV infection based on a HBsAg negative and HBcAb positive status. Prospective analysis of the effect of HBcAb on prognosis in 263 HCV carriers indicated that HBcAb had no effect on development of HCC, death caused by liver diseases and progression of hepatic fibrosis in HCV carriers.

The HBcAb positive rate is reported to be greater in HCV carriers than in healthy individuals. This study also showed a high HBcAb positive rate (53.3%, 213/400) in HCVAb positive subjects, in agreement with previous findings in Japan.[3,8] However, the HBcAb positive rate did not differ between HCV carriers and HCV RNA negative subjects, but did vary with age, with a higher rate in

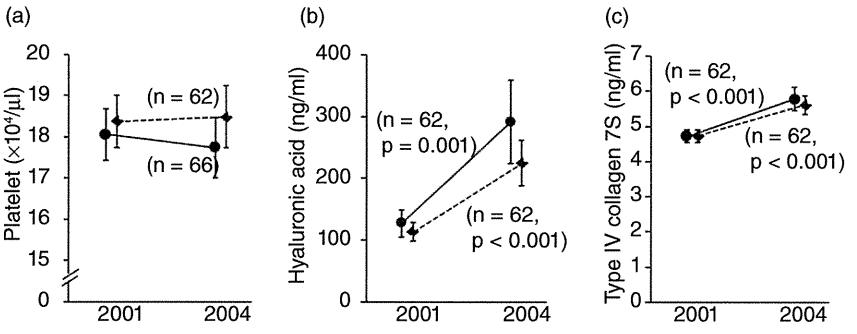

Figure 4 Fibrosis markers in HBcAb positive and HBcAb negative HCV carriers for whom data were obtained in 2001 and 2004. Platelet counts did not differ significantly between 2001 and 2004 (a). Serum hyaluronic acid (b) and type IV collagen 7S (c) levels significantly increased from 2001 to 2004, but the increases in these levels from 2001 to 2004 did not differ significantly between the HBcAb positive and HBcAb negative groups. HBcAb, antibody to hepatitis B core antigen; HCV, hepatitis C virus. •—•, HbcAb (+); •- -•, HbcAb (−).

elderly subjects. Thus, the incidence rate of occult HBV infection in HCV carriers might also have differed by age in the previous study, and the HBcAb positive rate in HCVAb negative patients living in the same area should be determined.

We found that a HBcAb positive status was not significantly related to development of HCC. There is some debate as to whether patients with HCV-related chronic liver disease develop HCC due to the presence of HBcAb or occult HBV infection. In a case–control study in 91 HCC patients with chronic hepatitis C and advanced hepatic fibrosis who did not respond to interferon (IFN) treatment (HCC group) and 182 patients with chronic hepatitis C who did not develop HCC (non-HCC group), Lok *et al.* found no significant differences in the prevalence of HBcAb and detection rate of HBV DNA in the liver between the two groups, and concluded that occult HBV infection was not involved in development of HCC in patients with HCV-related liver disease.[1] In a study of 140 chronic hepatitis C patients without HBsAg, Hasegawa *et al.* found no significant difference in the rate of development of HCC between 76 HBcAb positive and 64 HBcAb negative subjects.[16] In contrast, in a cohort study of 123 patients with HCV-related liver cirrhosis, Adachi *et al.* detected serum HBcAb in 78% of the subjects and the cumulative HCC development rate was significantly higher in the HBcAb positive group, with multivariate analysis indicating that a HBcAb positive status was an independent risk factor for development of HCC.[8] A prospective study in 275 patients with HCV-related liver cirrhosis indicated that HBcAb was the only factor associated with progression to HCC[3] and Shi *et al.* reported a relative risk of HCC of 2.83 for occult HBV infection in HCV-infected patients in a meta-analysis.[17] However, this meta-analysis focused on hospital-based populations and selection bias might have been present due to inclusion of the studies of Ikeda *et al.*[18] and Adachi *et al.*,[8] which targeted only liver cirrhosis patients. Another study showed a high rate of HCC due to HBcAb found in patients with HCV infection, but this rate was significant only in patients with liver cirrhosis.[3] In our study, the number of HCV carriers with liver cirrhosis was relatively low compared to previous reports, based on the platelet count measured in 1996. Therefore, occult HBV infection in HCV carriers may promote development of HCC in cases with liver cirrhosis, but occult HBV infection may have less effect on development of HCC in all HCV carriers.

Hepatitis B virus infection may promote hepatocarcinogenesis through integration of HBV DNA into the host genome, even in a case of occult HBV infection, and long-term expression of viral proteins such as HBx may be involved in hepatocarcinogenesis.[19] Thus, longer HBV infection including occult HBV infection may have a propensity for onset of HCC, and it is plausible that the risk for HCC development due to occult HBV infection in patients with HCV infection is higher in patients with liver cirrhosis compared to those with chronic hepatitis. In contrast, chronic hepatitis C progresses at varying rates and not all infected subjects necessarily develop liver cirrhosis and HCC. Factors such as sex and age are associated with development of liver cirrhosis and HCC in HCV carriers, but there may also be unidentified confounders such as host genetic factors, including single nucleotide polymorphisms. We speculate that confounders associated with liver cirrhosis are cofactors that contribute to induction of HCC in HCV carriers with occult HBV infection. Therefore, occult HBV infection in patients with HCV infection may be significantly high only in patients with liver cirrhosis and may exert only a precipitating effect on development of HCV-related HCC.[17]

There are many reports on occult HBV infection and development of HCC in HCV carriers, but few have examined the long-term prognosis. Our 10-year follow up indicated that a HBcAb positive status did not influence the long-term prognosis of patients with HCV carriers. In addition, this status did not influence liver-related death, such as that due to liver failure and rupture of esophageal varices. Age and ALT levels are known to influence the occurrence of HCC and liver-related mortality,[11,20,21] and this study also found that age of 65 years or older and ALT of 31 IU/L or more were independent risk factors for development of HCC and liver-related death. These results suggest that the subjects in this study were not a special population. Therefore, occult HBV infection may not influence the long-term prognosis of HCV carriers among community residents.

Several reports have suggested effects of occult HBV infection on progression of hepatic fibrosis and liver cirrhosis in patients with HCV-related chronic liver disease. In a study of 468 patients with chronic hepatitis C or liver cirrhosis, Matsuoka *et al.* reported that the degree of inflammatory cell infiltration in the liver was significantly higher in HBV DNA positive patients than in HBV DNA negative patients.[22] We did not perform histological evaluation in the current study, but there was no difference in ALT levels between HBcAb positive and HBcAb negative subjects; thus, the severity of hepatitis appears not to be influenced by HBcAb status. In addition, persistent hepatitis decreases the hepatic functional reserve, but we also found no difference in serum

albumin levels, an index of hepatic functional reserve, between the HBcAb positive and negative groups. These findings suggest that occult HBV infection does not influence hepatic functional reserve. However, we note that the number of subjects with low ALT levels was relatively high and the period for comparison of serum albumin levels was short (2001–2004) in our study. Thus, longer term observation in patients with more severe hepatitis is needed to clarify our findings.

Serum levels of hyaluronic acid and type IV collagen 7S, which are both serum hepatic fibrosis markers, were determined in 2001 and 2004 to evaluate hepatic fibrosis in HCV carriers. Neither marker showed a significant difference between the HBcAb positive and negative groups in either year. Evaluation of hepatic fibrosis using Fibroscan also indicated that hepatic fibrosis was not related to the presence or absence of HBcAb, and the significant increase in serum hepatic fibrosis markers from 2001–2004 was not related to occult HBV infection. These findings suggest that occult HBV infection does not influence hepatic fibrosis in HCV carriers.

There were some limitations in this study. First, the accuracy of the evaluation of occult HBV infection may be limited because HBV DNA in blood or liver tissue was not examined. However, serum HBcAb is a marker reflecting occult HBV infection,[4,5,23] and occult HBV infection can be detected even in a HBV DNA negative case. A future study is required to determine whether HBV DNA or HBcAb can accurately indicate occult HBV infection. Second, this study might not have had a sufficient number of patients with progression of hepatic fibrosis and active hepatitis. The numbers of HCV carriers with platelet count of more than 150 000/μL and albumin levels of 4 g/dL or more were 156 of 208 (75%) and 152 of 166 (92%), respectively, and only a few subjects progressed to liver cirrhosis. The number of subjects with ALT levels of 30 IU/L or less was 117 of 262 (45%), which may include many HCV carriers with persistent normal ALT.[24,25] Based on these data and the levels of hepatic fibrosis markers, the number of subjects with liver cirrhosis was small. Therefore, the 10-year follow-up period might not have been enough to show an influence of a HBcAb positive status on hepatic fibrosis and liver-related death, and further long-term observation is needed. Third, we did not exclude the effect of IFN treatment.[26] However, only a small number of patients were treated by IFN and there was no significant difference in IFN treatment history between the HBcAb positive and negative groups, which suggests that the results were not influenced by the effects of IFN treatment. Fourth, this study included a Japanese population with a relatively low mortality rate due to HCC.[11] Studies on cirrhosis and HCC indicate rates at 20 years after infection of 4–24% and 1–7%, respectively, depending on the study population and method of cohort recruitment.[27] Therefore, a meta-analysis of eligible studies is needed to evaluate whether occult HBV infection or a HBcAb positive status worsens the prognosis of HCV carriers. Fifth, the increased serum levels of hyaluronic acid seemed to indicate more severe hepatic fibrosis progression compared to those of type IV collagen 7S; and serum levels of hyaluronic acid in 2004 indicated advanced hepatic fibrosis, but type IV collagen 7S, platelet counts and elastography indicated mild hepatic fibrosis.[28–30] The reason for this difference is unclear, but serum levels of hyaluronic acid are likely to be affected by other factors such as arthritis.[31] In addition, platelet count, a hepatic fibrosis marker, did not change from 2001–2004. Therefore, progression of hepatic fibrosis appeared to be mild and occult HBV infection did not affect these changes. A histological evaluation is needed to confirm these findings.

In conclusion, a HBcAb positive status did not influence hepatic fibrosis, development of HCC or liver-related death in HCV carriers in a 10-year community-based cohort study. Our results indicate that occult HBV infection has no effect on the clinical course in HCV carriers.

ACKNOWLEDGMENTS

WE THANK MS Ayaka Hamabe and Ms Keiko Nakase for their technical assistance. This work was supported by a Grant-in-Aid for Research on Hepatitis and BSE from the Ministry of Health, Labour and Welfare of Japan (H24-Hepatitis-General-003) and a grant from the United States National Institutes of Health (no. CA87982).

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Hepatology Research

Hepatology Research 2013 doi: 10.1111/hepr.12226

Original Article

Hypophosphatemia in patients with hepatitis B virus infection undergoing long-term adefovir dipivoxil therapy

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Aim: The purpose of this study was to clarify the frequency of hypophosphatemia and other clinical features in patients with hepatitis B undergoing long-term therapy with adefovir dipivoxil (ADF).

Methods: Seventeen hepatitis B patients treated with a combination of lamivudine and ADF were analyzed. They were divided into two groups: patients who developed hypophosphatemia ($P < 2.5$ mg/dL) (group A) and those who did not (group B). The frequency of hypophosphatemia and other clinical features were retrospectively analyzed.

Results: There were six patients (35.3%) in group A. The treatment period was 57.3 ± 15.6 and 61.8 ± 25.7 months in groups A and B, respectively. No differences were found between the groups prior to treatment. Among the six patients in group A, osteomalacia was observed in two, while a pathological fracture of the scapula was found in one. Decreases in phosphate (96 weeks after starting ADF), estimated glomerular filtration rate (eGFR) (48 weeks) and uric acid (24 weeks) levels, and increases in creatinine and alkaline phosphatase were noted in group A.

Conclusion: Hypophosphatemia occurred in 35% of the patients under the long-term treatment with ADF. Although it was not possible to predict the decrease in phosphate before ADF therapy, decreases in uric acid and eGFR may be the early events relating to low phosphatemia.

Key words: adefovir dipivoxil, chronic hepatitis B, Fanconi's syndrome, hypophosphatemia, osteomalacia

INTRODUCTION

HEPATITIS B VIRUS (HBV), an incomplete circular DNA virus with approximately 3200 bases, induces a variety of liver diseases, such as acute hepatitis, fulminant hepatitis, chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Approximately 350 million people throughout the world are chronically infected with HBV,[1,2] with interferon and nucleoside/nucleotide analogs used for treating hepatitis B, of which lamivudine is the first nucleotide analog approved for hepatitis B patients. This drug has been reported to be effective for preventing progression from chronic hepatitis to liver cirrhosis or liver failure, and can prevent the occurrence of hepatocellular carcinoma.[3–6] However, the rate of emergence of resistant strains is reported to be high, and breakthrough hepatitis or viral infections occasionally occur.[7,8] Adefovir dipivoxil (ADF), known to be effective against lamivudine-resistant HBV, was approved in 2004 in Japan and is used in combination with lamivudine.[9,10] It has been reported that the incidence of ADV nephrotoxicity is dose-related, with the standard dose for HIV 30 mg daily or higher,[11,12] and the majority of patients receiving such a dose suffer from renal dysfunction. On the other hand, toxicity is not frequent in patients with HBV infection, for whom the approved dose of ADF is 10 mg daily.[13] Recently, hypophosphatemia and osteomalacia related to ADF have been reported.[14,15] In the present study, we analyzed the frequency and clinical features of hypophosphatemia in patients with hepatitis B undergoing long-term therapy with ADF.

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Received 10 March 2013; revision 6 August 2013; accepted 12 August 2013.

METHODS

Subjects

AMONG PATIENTS WITH hepatitis B treated with a nucleotide analog at our hospital between 2005 and 2012, 17 patients (13 men, four women, 61.3 ± 10.0 years old) were treated with lamivudine and ADF combination therapy for more than 12 months. All of these 17 patients were included in this study.

Methods

The patients were divided into two groups; those who developed ($P < 2.5$ mg/dL) (group A) and did not develop hypophosphatemia (group B). The frequency of hypophosphatemia and clinical features (age, sex, duration of treatment, clinical data) were analyzed. Liver function and renal function test results, and HBV markers were examined every 1–3 months. The hepatitis B e-antigen (HBeAg) and antibody against HBeAg (anti-HBe) were assayed using a chemiluminescence immunoassay. HBV DNA was assayed by transcription-mediated amplification (2005–2007.11) or real-time polymerase chain reaction (2007.12–2012). Estimated glomerular filtration rate (eGFR) (mL/min per 1.73 m^2) was calculated as follows: $194 \times (\text{creatinine [Cr]}^{-1.094}) \times (\text{age}^{-0.287}) \times (0.739 \text{ if female})$. Abdominal sonography or computed tomography examinations (and other imaging tests, if needed) were done every 6–12 months. The administrated dose of ADF was 10 mg/day, whereas it was reduced to 10 mg every other day when eGFR or Cr clearance was decreased between 30 to 49 mL/min followed by the criteria written in the interview form of ADF (Hepsera, GlaxoSmithKline, Tokyo, Japan).

The study was conducted in a retrospective manner. The purpose and details of the study were explained to the patients, and written informed consent was obtained from each.

Statistical analysis

Statistical analyses were performed using Student's *t*-test and Fischer's exact test using Prism 6 (GraphPad Software). $P < 0.05$ was regarded as significant.

RESULTS

SIX PATIENTS (35.3%) showed hypophosphatemia and were classified as group A. All six patients showed low phosphate levels, though one showed improvement after ADF was reduced to a half dose (10 mg/2 days). The other 11 patients were classified as group B. There were no differences regarding age, sex (male/female) and duration of treatment between the groups (55.0 ± 11.0 vs 58.6 ± 8.4 years, 5/1 vs 8/3, 57.3 ± 15.6 vs 61.8 ± 25.7 months, respectively) or for laboratory data obtained prior to treatment (Table 1). The duration of ADF therapy was more than 2 years in all except for one patient in group B. At the end of the observation period, laboratory data except for phosphate were not different between the groups (Table 2). Data at the end of the observation period are shown in Table 2. Besides the level of phosphate, significant difference was found in the rate of bone disease ($P = 0.04$). Though the differences were not significant, kidney stones or urine crystals was frequently found in group A (4/6 vs 3/11). Kidney stones or urinary crystals with occult hematuria were found in four patients in group A and three in group B. None of these patients had urinary stones or crystals before beginning ADF therapy. Mean level of alkaline phosphatase (ALP) was high (690.2 ± 725.2 vs 299.2 ± 117.9 IU) and that of uric acid was low (4.2 ± 2.0 vs 5.6 ± 1.6 IU) in group A.

Comparisons of data obtained at the start of ADF with those from the end of the observation period are shown in Table 3. In group A, a decrease in alanine aminotransferase (ALT) and eGFR, and increases in ALP and Cr were noted, while no significant differences except for ALT were seen in group B. Among the six patients in group A, osteomalacia was observed in two and a pathological fracture of the scapula was found in one. Profiles for these three patients are shown in Table 4, in whom the onset of bone disease was noted at 51, 59 and 42 months, respectively, after the start of ADF. Both patients with osteomalacia showed prominent hypophosphatemia and decreases in bone metal density in X-ray findings, while one had multiple rib fractures and the other had a fracture of a lower limb. Hypophosphatemia was found before the emergence of these fractures. None of the patients in group B showed bone disease.

Figure 1 shows the inverse correlation between ALP and phosphate at the end of the observation period, while the relationship between uric acid and phosphate is shown in Figure 2. Figure 3 shows the change of phosphate, uric acid, Cr and eGFR from the start of ADF administration. Gradual decreases in phosphate and eGFR, and gradual increases in Cr were found in group A. Although there was no significant difference in uric acid levels between before treatment and the end of the observation period, a significant decrease was found at 24, 48 and 96 weeks. Analysis of the sequential changes in those levels revealed that the first significant decrease in uric acid occurred at 24 weeks, while that of eGFR was

Table 1 Data obtained at beginning of ADF treatment

		Group A	Group B	*P*
Age	Years	60.2 ± 13.0	61.9 ± 8.6	0.54
Sex	Male : female	5:1	8:3	0.91
Body mass index		21.3 ± 3.0	22.2 ± 3.4	0.53
Diagnosis	CH : LC : after transplantation	2:3:1	9:2:0	0.51
Decreased ADF dose	+ : −	0:6	3:8	0.51
genotype	C : A (not determined)	3:1 (2)	7:0 (4)	0.36
HBeAg/Anti-HBe	+/− : −/+ : other	3:2:1	4:3:4	0.94
HBV-DNA	Log copy/mL	6.5 ± 1.4	7.2 ± 2.1	0.24
Diabetes Mellitus	+ : −	3:3	4:7	0.64
Hypertension	+ : −	3:3	3:8	0.60
Albumin	mg/dL	3.9 ± 0.7	3.7 ± 0.7	0.54
Total bilirubin	mg/dL	3.6 ± 6.2	1.1 ± 0.7	0.59
AST	IU	146.0 ± 202.2	134.7 ± 120.0	0.53
ALT	IU	198.5 ± 287.6	168.5 ± 204.4	0.54
White blood cells	/μL	5178 ± 2631	4607 ± 1335	0.59
Hemoglobin	g/dL	13.8 ± 1.1	13.6 ± 2.1	0.56
Platelets	$\times 10^4/\mu L$	13.4 ± 5.3	14.8 ± 6.1	0.58
ALP	IU	285.2 ± 61.9	330.3 ± 167.2	0.59
Uric acid	mg/dL	5.2 ± 1.5	5.3 ± 1.7	0.55
BUN	mg/dL	13.4 ± 4.3	17.3 ± 6.7	0.40
Cr	mg/dL	0.6 ± 0.2	0.9 ± 0.3	0.07
eGFR	mL/min per 1.73 m^2	99.6 ± 24.9	67.7 ± 24.8	0.05
Calcium	mg/dL	9.2 ± 0.9	9.0 ± 0.7	0.61
Phosphate	mg/dL	3.2 ± 0.9	3.4 ± 0.5	0.53

ALP, alkaline phosphatase; ALT, alanine aminotransferase; anti-HBe, hepatitis B virus e-antibody; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CH, chronic hepatitis; Cr, creatinine; eGFR, estimated glomerular filtration rate; HBeAg, hepatitis B virus e-antigen; LC, liver cirrhosis; N.S., not significant.

at 48 weeks and of phosphate was at 96 weeks in group A. In group B, significant changes were not found until 48 weeks in all of these test items; significant decrease in eGFR and increase in Cr were barely found after 72 weeks. Figure 4 shows the changes in these levels in a representative subject in group A. Gradual changes were observed, with changes in Cr, uric acid and eGFR.

DISCUSSION

LEVEL OF PHOSPHATE is affected by many factors such as endocrine factors, complicated diseases and environmental factors. Thyroid hormone and growth hormones as well as parathyroid hormone (PTH) are known to relate with the level of phosphate. Intestinal diseases including malabsorption syndrome may cause low phosphatemia due to disturbance of absorption or exceed secretion of phosphate from the intestine. Several medicines such as sorafenib may also cause low phosphatemia. In the present study, level of PTH or other hormones was not assayed in all cases, but no patients in the present study showed symptoms of the above diseases, and none of them had taken other medicines inducing low phosphatemia. Therefore, low phosphatemia in the present study was supposed to be caused by administration of ADF.

In the present study, hypophosphatemia occurred in 35.3% of patients treated with ADF, which was higher than that in a previous report (6.5%).[16] This difference may be due to the different periods of ADF therapy, as that was approximately 60 months in the present study and 48 weeks in the other report. Therefore, the risk of hypophosphatemia during long-term therapy may be higher than previously thought.

The mechanism of hypophosphatemia development in patients being treated with ADF has been reported to be related to toxicity of the drug in proximal convoluted tubules of the kidneys. Injury to those tubules causes a disturbance of reabsorption as well as increased excretion of amino acids, sugar, uric acid, bicarbonate and

Table 2 Data obtained at end of observation period

		Group A	Group B	*P*
ADF administrated time	Months	57.3 ± 15.6	61.8 ± 25.7	0.49
Total amount of administrated ADF	mg	15800.0 ± 5914.4	16213.6 ± 8812.9	0.57
Decreased dose of ADF	+ : −	1:5	1:10	0.75
HBeAg/anti-HBe	+/− : −/+ : other	0:3:3	4:3:4	0.61
HBV DNA	≥2.1 : <2.1 : undetected	0:3:3	3:3:5	0.73
Bone disease	+ : −	3:3	0:11	0.04
Kidney stones or urine crystals	+ : −	4:2	3:8	0.16
Albumin	mg/dL	4.3 ± 0.8	4.2 ± 0.7	0.43
Total bilirubin	mg/dL	1.5 ± 1.8	0.8 ± 0.5	0.52
AST	IU	32.7 ± 28.3	27.2 ± 15.0	0.54
ALT	IU	21.0 ± 14.3	20.8 ± 12.6	0.57
White blood cells	/μL	5225 ± 1372	4305 ± 1236	0.21
Hemoglobin	g/dL	14.2 ± 1.8	13.8 ± 2.1	0.57
Platelets	$\times 10^4/\mu L$	14.4 ± 5.2	15.3 ± 5.5	0.55
ALP	IU	690.2 ± 725.2	299.2 ± 117.9	0.15
Uric acid	mg/dL	4.2 ± 2.0	5.6 ± 1.6	0.17
BUN	mg/dL	14.5 ± 3.4	19.1 ± 6.0	0.11
Cr	mg/dL	0.9 ± 0.3	1.0 ± 0.2	0.29
eGFR	mL/min per 1.73 m^2	67.3 ± 21.8	57.6 ± 19.0	0.21
Calcium	mg/dL	9.2 ± 0.9	9.4 ± 0.6	0.57
Phosphate	mg/dL	2.1 ± 0.7	3.2 ± 0.3	0.007

ADF, adefovir dipivoxil; ALP, alkaline phosphatase; ALT, alanine aminotransferase; anti-HBe, hepatitis B virus e-antibody; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cr, creatinine; eGFR, estimated glomerular filtration rate; HBeAg, hepatitis B virus e-antigen.

phosphate in urine, resulting in renal tubular acidosis, low uric academia and hypophosphatemia. These conditions are designated as Fanconi's syndrome and that due to ADF has been reported.[17,18] Prolongation of these conditions results in osteomalacia and pathological fractures, while electrolyte abnormalities and osteopenia cause such symptoms as muscle weakness, fatigue, bone pain and pseudofractures.[19–21] As a result, osteomalacia develops and patient quality of life is reduced.

Adefovir dipivoxil toxicity appears to be related to organic anion transporter (hOAT1)-mediated cellular accumulation and transport of fluorescein methotrexate with multidrug resistance-associated protein2 (Mrp2). *In vitro* studies have demonstrated that overexpression

Table 3 Comparison of data obtained at beginning of ADF treatment and end of observation period

		Group A		*P*	Group B		*P*
		Beginning	End of observation		Beginning	End of observation	
ALT	IU	198.5 ± 287.6	21.0 ± 14.3	0.03	168.5 ± 204.4	20.8 ± 12.6	0.004
ALP	IU	285.2 ± 61.9	690.2 ± 725.2	0.03	330.3 ± 167.2	299.2 ± 117.9	0.55
Uric acid	mg/dL	5.2 ± 1.5	4.2 ± 2.0	0.57	5.3 ± 1.7	5.6 ± 1.6	0.53
BUN	mg/dL	13.4 ± 4.3	14.5 ± 3.4	0.70	17.3 ± 6.7	19.1 ± 6.0	0.32
Cr	mg/dL	0.6 ± 0.2	0.9 ± 0.3	0.04	0.9 ± 0.3	1.0 ± 0.2	0.39
eGFR	mL/min per 1.73 m^2	99.6 ± 24.9	67.3 ± 21.8	0.05	67.7 ± 24.8	57.6 ± 19.0	0.49
Calcium	mg/dL	9.2 ± 0.9	9.2 ± 0.9	0.90	9.0 ± 0.7	9.4 ± 0.6	0.35
Phosphate	mg/dL	3.2 ± 0.9	2.0 ± 0.6	0.02	3.4 ± 0.5	3.2 ± 0.3	0.67

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cr, creatinine; eGFR, estimated glomerular filtration rate.

Table 4 Patients with bone disease

	Patient 1	Patient 2	Patient 3
Age	51	70	36
Sex	Male	Female	Male
Diagnosis of liver disease	Liver cirrhosis	Chronic hepatitis	Liver cirrhosis
Start of lamivudine	Aug-2004	Feb 2006	Jan 2005
Dose of lamivudine	100 mg daily	100 mg daily	100 mg daily
Start of adefovir	May 2005	Dec 2006	Sep 2006
Dose of adefovir	10 mg daily	10 mg daily	10 mg daily
Hypophosphatemia	May 2007	Dec 2008	Mar 2008
Kidney stones or urine crystals with occult hematuria	Oct 2008 (41 months later)	Oct 2008 (22 months later)	Jan 2010 (40 months later)
	Calcium phosphate and calcium oxalate with occult hematuria	Calcium oxalate with occult hematuria	Renal stones
Bone disease	Aug 2009 (51 months later) Osteomalacia	Nov 2011 (59 months later) Osteomalacia	Mar 2010 (42 months later) Scapula fracture

of hOAT1 in Chinese hamster ovary cells exposed to ADF and cidofovir resulted in increases in intracellular concentrations of both drugs.[22–25]

In the present study, the clinical features and laboratory data for groups A and B prior to ADF therapy were similar, and it was not possible to predict the emergence of hypophosphatemia in individual patients before beginning treatment. However, decreases in uric acid and eGFR, and an increase in Cr was found in patients with low phosphatemia. In addition, reverse correlation was found between ALP and phosphate. Elevation of ALP was supposed to be originated from bone due to secondary elevation of PTH followed by low phosphatemia. On the other hand, phosphate and uric acid showed positive correlation. These two substances are reabsorbed from the proximal tubule of the kidney. Although the transporters of phosphate and uric acid in the proximal tubule are different, decrease of the both substances was supposed to be due to the damage of the proximal tubule by ADF. It should be noted that decrease in serum phosphate was significant at 96 weeks after beginning ADF, whereas significant decreases in uric acid and eGFR, and an increase in Cr occurred at 24, 48 and 72 weeks, respectively. These data indicated that decreases in uric acid and eGFR preceded the decrease of phosphate. It might be suspected that the screening for those parameters may be useful to predict hypophosphatemia or following bone complications. However,

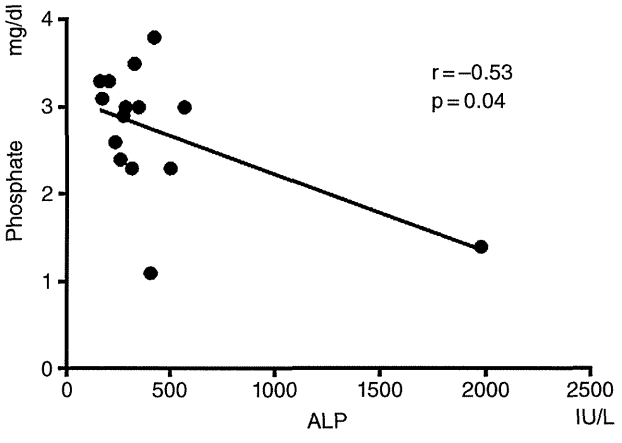

Figure 1 Relationship between alkaline phosphatase (ALP) and phosphate (data obtained at end of observation period).

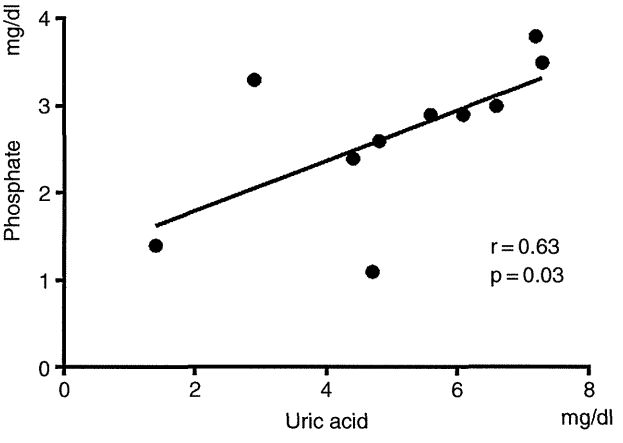

Figure 2 Relationship between uric acid and phosphate (data obtained at end of observation period).