clearance of HCV viremia during combination therapy has been reported to be a good indicator for higher SVR rate. As expected, rapid and early virological responses were seen in 80% of patients with high sensitivity but were found in only 40% of patients with intermediate or low sensitivity. Null or very late virological responders were quite rare in the high-sensitivity group. Because all rapid and early virological responders achieved SVR, patients who are judged to have high sensitivity are strongly recommended to take Peg-IFN and RBV combination therapy.

It is noteworthy that actual SVR rates were similarly low in the low-sensitivity group and high in the high-sensitivity group irrespective of total doses of Peg-IFN and RBV administered, but were significantly associated with total dose in the intermediate-sensitivity group. Thus, efforts to maintain at least standard administration doses of Peg-IFN and RBV are important to achieve higher SVR rates in patients who have intermediate sensitivity to combination therapy.

Several pretreatment factors other than ours have been reported to be associated with virological response to IFN and RBV combination therapy in patients with genotype 1b HCV infection, including serum levels of low-density lipoprotein cholesterol and insulin resistance. However, the results obtained here are valuable because the estimated sensitivities to combination therapy are very closely associated with actual sensitivities. Our algorithm cannot be applied to other populations directly because clinical backgrounds, such as distribution of body weight, differ globally. However, a number of mutations in the ISDR sequence of the hepatitis C virus have been shown to be associated with response to IFN therapy in a worldwide meta-analysis reported by Pascu et al.[30] Immunological factors, such as Th1/Th2 imbalance, have also been reported to be associated with treatment response in the world.[16,17] As such, the current study is applicable on a global scale because it clearly shows that a combination of viral and host factors, including those of immunological nature, are effective for predicting the response to combination therapy before it is started.

In conclusion, in Japanese patients from Nagano, SVR rates of Peg-IFN and RBV combination therapy can be accurately predicted using the pretreatment factors of ISDR mutations, Th1/Th2 ratios, body weights, and neutrophil counts. This being established, future prospective trials are required to validate our results in other regions of Japan and in other countries.

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Appendix A

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CLINICAL REVIEW

History and prevention of de novo hepatitis B virus-related hepatitis in Japan and the world

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Abstract Hepatitis B virus (HBV) replication has been shown to persist at low levels in the liver for decades, even in patients with resolved HBV infection. In these cases, reactivation of HBV and ensuing hepatitis during or after cytotoxic or immunosuppressive therapy is now recognized as de novo HBV-related hepatitis. The occurrence of de novo HBV-related hepatitis has become more frequent after the introduction of rituximab for the treatment of hematological disorders, such as malignant lymphomas. More alarmingly, reactivation can lead to fatal fulminant hepatic failure, indicating a need to establish guidelines to prevent the occurrence of de novo HBV-related hepatitis. It is possible that lamivudine prophylaxis and close surveillance of serum HBV DNA are effective in this regard. However, such measures are currently not available to hepatitis B surface antigen (HBsAg)-negative patients in Japan. A preliminary guideline for preventing HBV reactivation during and after cytotoxic or immunosuppressive therapies was made in 2008 by two collaborative study groups from the Japanese Ministry of Health, Labour, and Welfare, including measures not only for HBV carriers, but also for patients with resolved HBV infection. Since this recommendation is a tentative one, further testing and improvements are being planned.

Keywords Hepatitis B virus · De novo hepatitis · Reactivation · Immunosuppression · Prevention

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History and prevention

Approximately 3 billion people have been exposed to the hepatitis B virus (HBV), and there are an estimated 350 million chronic carriers worldwide [1–3]. The clearance of circulating hepatitis B surface antigen (HBsAg) and appearance of antibody to HBsAg (anti-HBs) with normalization of liver function were generally considered as evidence of complete clearance of HBV from hosts until the early 1990s. Since then, it has been shown that HBV replication persists at low levels in the liver and peripheral blood mononuclear cells for decades, even in HBsAg-negative patients with resolved HBV infection [4–6]. In such patients, HBV replication is suppressed by immune responses to HBV, including specific cytotoxic T lymphocyte (CTL)-mediated responses [4].

HBV reactivation in patients with resolved infection is being reported in increasing numbers because the number of people undergoing strong immunosuppressive therapy is increasing worldwide, especially patients with malignant neoplasms, autoimmune disorders, and following transplantation for prevention of rejection. In those patients with resolved HBV infection, reactivation of HBV and ensuing hepatitis is recognized as de novo HBV-related hepatitis, which sometimes leads to fulminant hepatic failure (FHF) and is thus becoming an alarming, well-recognized complication of immunosuppressive therapy that needs further attention [7–9].

Wands et al. [10] reported that reseroconversion from anti-HBs to HBsAg was observed in 5 (13%) of 40 patients with myeloproliferative or lymphoproliferative disorders who received cytotoxic chemotherapy in 1975. This might have been the first report suggesting an association between HBV reactivation and chemotherapy in patients who showed the serological markers of resolved HBV infection.

However, the clinical significance of these phenomena was not clear at that time because our knowledge regarding occult HBV infection was limited. In 1991, Lok et al. reported a prospective study on hepatitis associated with HBV reactivation in patients with malignant lymphoma who received cytotoxic chemotherapy [11]. In their study, HBV reactivation and resulting hepatitis was common in patients who were HBV carriers, but rare (2%) in patients with resolved HBV infection. On the other hand, de novo HBV hepatitis was relatively frequent (14–50%) in patients with hematological disorders who received allogenic bone marrow transplantation [12–16]. This difference in frequency could be attributed to the difference in extent of immunosuppression between the treatments; generally, immune responses are suppressed more profoundly in patients with allogenic bone marrow transplantation than in those with ordinary cytotoxic chemotherapy.

The recent occurrence of HBV reactivation hepatitis in the treatment of hematological disorders, such as malignant lymphomas, has drawn the attention of both hepatologists and hematologists [7, 9]. It is considered to be caused mainly by the introduction of rituximab, which is used mainly for the treatment of B-cell-type malignant lymphomas. Accordingly, the US Food and Drug Administration reported a possible relationship between FHF and rituximab use in October 2004. Rituximab is a genetically engineered chimeric murine/human monoclonal antibody against the CD20 antigen found on the surface of normal and malignant B lymphomas and is used alone or in combination with cytotoxic chemotherapeutic drugs [17, 18]. Dervite et al. [19] first reported a possible relationship between HBV reactivation and rituximab use in a patient with resolved HBV infection in 2001. Following that report, several cases of de novo HBV-related hepatitis after treatment with rituximab that proved fatal were reported [20–24]. B cells may act as antigen-presenting cells and prime CTL responses in HBV infection. Rituximab induces profound and durable B cell depletion to an extent of 0% CD20-positive cells, thus possibly enabling reactivation of CTL-suppressed HBV replication to occur.

Hui et al. [25] evaluated the risk of developing de novo HBV-related hepatitis after chemotherapy in Hong Kong. They prospectively followed 244 patients with lymphomas who were negative for HBsAg for a median period of 12.4 months. In their report, eight (3.3%) patients developed de novo HBV-related hepatitis. These eight patients were presumed to have occult HBV infection because they were found to be positive for at least one anti-HBs and for anti-HBc. Of these patients, three developed FHF, one of whom died. Multivariate analysis showed that de novo hepatitis was independently associated with higher risk of FHF, with a relative risk of 29.9. A risk factor for developing de novo hepatitis was the use of a rituximab plus steroid-containing regime. In addition, elevation of HBV DNA, HBsAg, and alanine aminotransaminase (ALT) values were seen after finishing chemotherapy. Since a 100-fold increase in serum HBV DNA preceded the onset of de novo hepatitis by a median period of 18.5 (range 12–28) weeks, Hui recommended close surveillance for such an increase so that antiviral therapy could be initiated as quickly as possible. On the other hand, Liu et al. suggested the possibility that short-term lamivudine prophylaxis during chemotherapy for patients with occult HBV infection is more cost effective than close surveillance of serum HBV DNA [26]. Further studies are required to clarify this matter.

De novo HBV-related hepatitis after orthotopic liver transplantation was first reported by Douglas et al. in 1993 [27]. Uemoto et al. [28] clarified that HBV strains found in a donor liver before transplantation were the same as those found in a corresponding recipient who developed de novo hepatitis by comparing nucleotide sequences in the HBV genome. Rokuhara et al. [29] also showed that those two strains of HBV were identical by determining the full nucleotide sequence of the HBV genome. According to reports so far, the incidence of de novo hepatitis ranges from 33% to 94% when livers are transplanted from donors with anti-HBc, and from 0% to 0.5% when livers are transplanted from donors without [27–32] indicating that de novo HBV-related hepatitis in liver transplantation recipients is closely associated with occult HBV infection in donor livers. Since the occurrence of de novo hepatitis is quite frequent when donors show serological markers of resolved HBV infection, it may be prevented by the administration of hepatitis B immunoglobulins and nucleot(s)ide analogues in combination [33].

The state of de novo HBV-related hepatitis in Japan was surveyed in 2005 by a group directed by Dr. Kumada (Toranomon Hospital) from the Ministry of Health, Labour, and Welfare of Japan (study for standardization of treatment of viral liver diseases including liver cirrhosis) [34]. In this retrospective study, a total of 55 patients with de novo HBV-related hepatitis were seen between January 2000 and December 2004 in 90 hospitals. During the same period, approximately 1,000 patients with typical acute hepatitis B were diagnosed in those hospitals. Among the 55 patients with de novo HBV-related hepatitis, 27% developed FHF, compared with only 7% of patients with acute hepatitis B. It is noteworthy that mortality was as high as 100% in patients with de novo hepatitis who developed FHF [8]. Taken together, it is evident that de novo HBV-related hepatitis with a strong tendency to develop into FHF with high mortality is an important issue that needs to be addressed.

There seem to be no official guidelines for preventing de novo HBV-related hepatitis occurring during or after cytotoxic or immunosuppressive therapy in the world. The American Association for the Study of Liver Diseases has

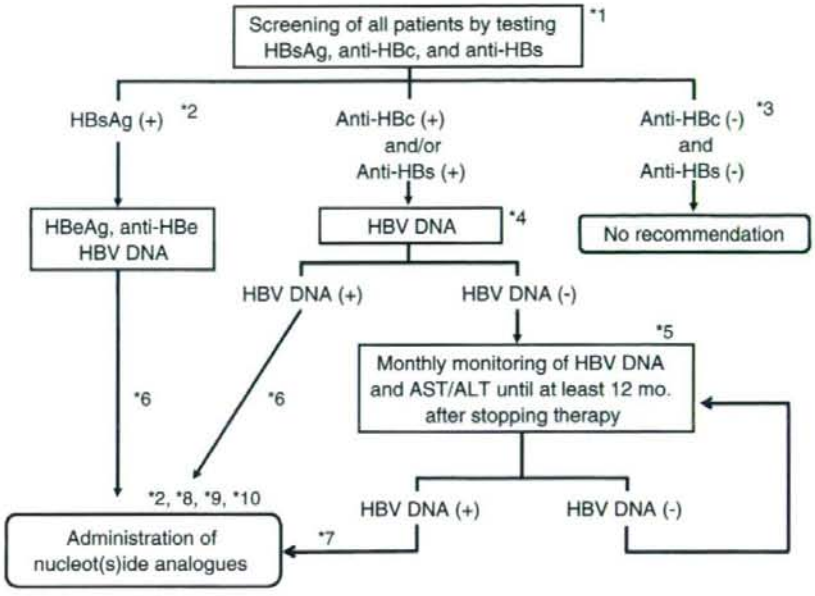

Fig. 1 Recommendation for preventing hepatitis B due to reactivation of HBV during and after immunosuppressive or cytotoxic therapies. The prophylactic administration of nucleot(s)ide analogues recommended here is not completely based on evidence, and thus does not necessarily guarantee the prevention of fulminant hepatic failure. *1 Measurement using chemiluminescence immunosorbent assay is recommended. *2 Consultation with a hepatologist is recommended. *3 It is possible that anti-HBc and anti-HBs becomes undetectable during immunosuppressive or cytotoxic therapies. Additional measurement of HBV DNA is recommended in these cases. *4 HBV DNA is recommended to be tested by methods having the highest sensitivity available. *5 A high risk of developing de novo HBV-related hepatitis should be noted in patients who receive a rituximab plus steroids regime or in those who underwent hematopoietic cell transplantation. Fuldarabine can suppress immune responses profoundly and thus its use requires attention; however, its potential for causing reactivation of HBV is currently unknown. *6 Administration of nucleot(s)ide analogues is recommended as early as possible before starting immunosuppressive or cytotoxic therapies. *7 Administration of nucleot(s)ide analogues is recommended as soon as possible when HBV DNA becomes detectable during immunosuppressive or cytotoxic therapies. *8 Entecavir is recommended among nucleot(s)ide analogues. *9 In HBV-carrier patients, discontinuation of nucleot(s)ide analogues can be considered when patients meet the conditions shown in the guidelines for the treatment of hepatitis B patients prepared by Dr. Kumada and the Ministry of Health, Labour, and Welfare of Japan in 2008. In patients with resolved HBV infection at screening, discontinuation of nucleot(s)ide analogues can be considered when patients meet all of the following conditions: (1) the period after discontinuing immunosuppressive or cytotoxic therapies is at least 12 months, (2) ALT levels are within normal range during this period, and (3) HBV DNA levels are under detection limits during this period. *10 Patients should be followed carefully for 12 months after discontinuing nucleot(s)ide analogues. If serum HBV DNA becomes detectable during the follow-up period, administration of nucleot(s)ide analogues should be recommenced as soon as possible

published the antiviral prophylaxis for HBV carriers, but not for patients with resolved HBV infection [35]. It is possible that nucleot(s)ide analogue prophylaxis and close surveillance of serum HBV DNA are effective to prevent occurrence of de novo HBV-related hepatitis [25, 26]. However, such measures are currently not available to HBsAg-negative patients in Japan. The incidence of de novo HBV-related hepatitis is expected to increase in the future with the advent of stronger immunosuppressive and cytotoxic drugs, such as rituximab. As de novo hepatitis sometimes causes fatal FHF that cannot be controlled with nucleot(s)ide analogues after the onset of hepatitis, guidelines to prevent the occurrence of reactivation are needed.

A preliminary recommendation for preventing HBV reactivation during and after cytotoxic or immunosuppressive therapies was prepared in 2008 by two study groups from the Ministry of Health, Labour, and Welfare of Japan in collaboration: the study group for the standardization of treatment of viral liver diseases including liver cirrhosis directed by Dr. Kumada (Toranomon Hospital), and the study group for intractable liver and biliary tract diseases directed by Dr. Tsubouchi (Kagoshima University). This recommendation includes measures not only for HBV carriers, but also for patients with resolved HBV infection, as shown in Fig. 1. Although the guideline is tentative and may need future amendments, it nonetheless represents a first step in addressing the increasing global problem of de novo HBV-related hepatitis.

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BRIEF REPORT

Mortality Secondary to Fulminant Hepatic Failure in Patients with Prior Resolution of Hepatitis B Virus Infection in Japan

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Hepatitis B virus (HBV) reactivation in patients with resolved HBV infection was found in 23 (4%) of 552 newly hepatitis B surface antigen–positive patients in Japan. Because one-fourth of cases develop into fulminant hepatic failure and mortality is 100%, management of HBV reactivation in patients with resolved HBV infection should be discussed.

Reactivation of hepatitis B virus (HBV) is becoming a well-recognized complication in patients with chronic HBV infection who are undergoing cytotoxic chemotherapy or immunosuppressive therapy [1–5]. HBV reactivation has a variety of manifestations, ranging from subclinical increases in transaminase activity to severe and potentially fatal fulminant hepatic failure (FHF). Because clinical studies have demonstrated that lamivudine therapy reduces the rate of HBV reactivation and mortality [6–9], prophylactic antiviral therapy is advised for HBV carriers at the onset of chemotherapy [10].

The clearance of hepatitis B surface antigen (HBsAg) and the appearance of antibody to HBsAg, with normalization of liver function, is generally accepted as evidence of clinical and serologic recovery from acute hepatitis B. However, HBV replication has been shown to persist at low levels in the liver for decades [11–13], which may explain the recent increase in the rate of HBV reactivation in patients with resolved infection during or after chemotherapy and transplantation [1, 5, 14–16]. Although reactivation led to FHF and even death in some cases [17–22], the incidence of and mortality associated with HBV reactivation have not been fully clarified in this context. Recently, a prospective study [23] from Hong Kong revealed that 3.3% of HBsAg-negative patients developed HBV reactivation after chemotherapy. In Japan, because ~20% of individuals are positive for at least 1 HBV marker [24], HBV reactivation during or after immunosuppressive treatment may become a critical issue in the near future. Thus, we investigated the mortality associated with and prevalence and clinical significance of HBV reactivation in Japanese patients with resolved HBV infection in a multicenter, cross-sectional study.

Methods. In 2005, we sent a questionnaire to 230 hospitals certified by the Japan Society of Hepatology; this included questions about patients who had become newly positive for serum HBsAg from January 2000 through December 2004 [25]. A total of 1239 patients were registered by 93 hospitals (40%). Of those patients, 55 were recorded as having experienced HBV reactivation after having resolved HBV infection, and the remaining 1184 patients were classified as having acute hepatitis B. Sixty-three (68%) of 93 hospitals responded to a second survey and provided information on 552 patients enrolled in this study; 23 of these patients developed HBV reactivation, and 529 had acute hepatitis B.

HBV reactivation was defined (according to a slight modification of the report by Hui et al. [23]) as a decrease in the level of antibody to HBsAg that was associated with the reappearance of HBsAg, a 3-fold elevation of serum alanine aminotransferase (ALT) level above normal, and detection of HBV DNA in serum during or after chemotherapy. The diagnoses of acute hepatitis B and FHF were defined as reported elsewhere [26]. Patients with other liver diseases were excluded. Serum HBV markers were determined as reported elsewhere [26]. Serum levels of HBV DNA were determined with use of Amplicor HBV Monitor kits (Roche Diagnostics) at each hospital when the patients were admitted. HBV genotypes were determined with use of the PCR-invader method, with genotype-specific probes [27]. This study was approved by the ethics committees of appropriate institutional review boards. Informed consent was obtained from each patient in accordance with the Helsinki Declaration.

The Mann-Whitney U test was used to analyze continuous variables. The χ^2 test with Yate's correction was used for analysis of categorical data. In cases in which the number of patients was <5, Fisher's exact test was used. $P \leq .05$ was considered to

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be statistically significant. Statistical analyses were performed using SPSS, version 15.0J (SPSS).

Results. We first compared the demographic, clinical, and virologic features of the 23 patients who experienced HBV reactivation with those of the 529 patients with acute hepatitis B (table 1). The reactivation group had a significantly higher median age and median serum HBV DNA level ($P < .001$) and significantly lower peak ALT and albumin levels ($P < .001$). Although HBV genotype was not determined for one-half of the patients with acute hepatitis B, marked differences in the distribution of genotypes were seen; HBV type A occurred less frequently ($P = .003$) among patients with HBV reactivation than among those with acute hepatitis. However, HBV type B occurred more frequently among patients with HBV reactivation ($P < .001$).

FHF was more common among patients with HBV reactivation than among those with acute hepatitis ($P = .048$). Of the 23 cases of HBV reactivation, 6 (26%) resulted in liver-related death, 11 (48%) resolved, and 6 (26%) led to chronic hepatitis B. In contrast, of the 529 cases of acute hepatitis B, 490 (93%) were self-limited, 16 (3%) became chronic, and 21 (4%) resulted in death. These results revealed that liver-related mortality was significantly higher in the group with HBV reactivation than in the group with acute hepatitis ($P < .001$).

We then compared the clinical features of FHF between the groups (table 2). Patients with HBV reactivation had a higher median age, significantly lower peak ALT levels ($P = .006$), higher HBV DNA levels ($P = .035$), and higher mortality ($P = .031$) than did patients with acute hepatitis B.

Malignant lymphoma–associated morbidity was significantly higher among patients with HBV reactivation who developed FHF than among those who did not develop FHF (table 3). A rituximab-containing treatment regimen was administered to all patients who experienced FHF, compared with only 4 (22%) of 18 patients who did not experience FHF ($P = .004$). Lamivudine was administered to 16 (89%) of 18 patients who did not experience FHF and to all patients who experienced FHF at 7 and 20 days after hospital admission, respectively; this suggests that lamivudine treatment could not prevent FHF after HBV reactivation. Eventually, liver-related mortality occurred exclusively in patients who experienced FHF. There were no statistically significant differences between the 2 subgroups regarding HBV markers.

Discussion. Although a prospective study by Hui et al. [23] revealed that the incidence of HBV reactivation among HBsAg-negative patients after chemotherapy was 3.3%, there are no data available on HBV reactivation in Japan. In our nationwide cross-sectional study, a total of 552 newly HBsAg-positive patients were registered from 63 tertiary care hospitals. Overall, HBV reactivation was found in 4% of patients with resolved infection after chemotherapy. Serum and liver samples were not available before chemotherapy for most of these patients; therefore, we were unable to prove specifically whether reactivation was a result of occult or acute HBV infection. However,

Table 1. Demographic and clinical characteristics of patients with hepatitis B virus (HBV) reactivation, compared with those of patients with acute hepatitis B.

Characteristic	Patients with HBV reactivation	Patients with acute hepatitis B	*P*
Age, median years (95% CI)	63 (39–83)	33 (19–64)	<.001
Male sex	14/23 (61)	374/529 (71)	NS
Peak ALT level, median IU/L (95% CI)	929 (137–2441)	2300 (299–6626)	<.001
Peak bilirubin level, median mg/dL (95% CI)	10.3 (0.3–58.6)	6.4 (1.0–23.7)	NS
Lowest albumin level, median g/dL (95% CI)	3.2 (2.1–3.7)	3.6 (2.7–4.2)	<.001
Most prolonged PT%, median % (95% CI)	65.0 (10.2–121.4)	75.0 (11.0–103.1)	NS
HBV DNA level, median log copies/mL (95% CI)	7.5 (4.0 to >7.6)	5.5 (2.6 to >7.6)	<.001
Genotype			
A	0/19 (0)	57/232 (25)	.003
B	8/19 (42)	27/232 (12)	<.001
C	11/19 (58)	141/232 (61)	NS
Other	0/19 (0)	7/232 (3)	
Treatment			
Lamivudine	20/23 (87)	118/529 (22)	<.001
IFN	5/23 (22)	12/529 (2)	<.001
Fulminant hepatic failure	5/23 (22)	45/529 (9)	.048
Liver-related death	6/23 (26)	21/529 (4)	<.001

NOTE. Data no. (%) of patients, unless otherwise indicated. ALT, alanine aminotransferase; NS, not statistically significant; PT, prothrombin time.

Table 2. Demographic and clinical characteristics of patients with hepatitis B virus (HBV) reactivation who experienced fulminant hepatic failure (FHF), compared with those of patients with acute hepatitis B who experienced FHF.

Characteristic	Patients with FHF		P
	With HBV reactivation	With acute hepatitis B	
Age, median years (95% CI)	63 (47–64)	48 (18–72)	.029
Male sex	3/5 (60)	26/45 (58)	NS
Peak ALT level, median IU/L (95% CI)	907 (359–1823)	5995 (589–11,858)	.006
Peak bilirubin level, median mg/dL (95% CI)	20.8 (10.2–45.7)	9.9 (4.9–30.5)	.099
Lowest albumin level, median g/dL (95% CI)	2.6 (2.1–3.0)	2.9 (1.9–3.9)	NS
Most prolonged PT%, median % (95% CI)	22.0 (8.7–32.3)	16.0 (0.2–37.0)	NS
HBV DNA level, median log copies/mL (95% CI)	7.6 (5.6 to >7.6)	5.7 (2.6 to >7.6)	.035
Genotype			
A	0/5 (0)	2/16 (13)	NS
B	1/5 (20)	3/16 (19)	NS
C	4/5 (80)	11/16 (69)	NS
Received lamivudine treatment	5/5 (100)	29/45 (81)	NS
Liver-related death	5/5 (100)	21/45 (47)	.031

NOTE. Data are no. (%) of patients, unless otherwise indicated. ALT, alanine aminotransferase; NS, not statistically significant; PT, prothrombin time.

because all patients were negative for HBsAg and positive for antibody to hepatitis B core antigen before treatment, we presumed that reactivation was occult in nature.

In our study, patients who experienced HBV reactivation were significantly older and had lower serum albumin levels, compared with patients with acute hepatitis B. The immune status of many patients may have been further decreased by cytotoxic chemotherapy. Approximately 20% of the patients who experienced HBV reactivation developed FHF. Surprisingly, mortality was 100%, implying that FHF in these cases is severe. Both the prevalence of and mortality associated with FHF were significantly higher among patients who experienced HBV reactivation than among those with acute HBV infection. Although the group with HBV reactivation also had lower albumin levels at the onset of lamivudine therapy, the development of FHF could not be predicted from this study. Thus, it is crucial to prevent FHF in patients with HBV reactivation with use of agents other than—or complimentary to—lamivudine. Unfortunately, preemptive therapy is not recommended because of the difficulties in detecting reactivation. Hui et al. [23] recommended monthly testing of HBV DNA levels and immediate antiviral therapy when levels are 100-fold the levels before chemotherapy. However, this strategy is still controversial [28, 29] and needs testing in a randomized study.

A recent study revealed that HBV type Bj and G1896A mutations were independently associated with a fulminant outcome in patients with acute HBV infection [30]. However, HBV genotype, serum HBV DNA level, or mutations in G1896A or A1762T/G1764A did not influence the development of FHF in patients who experienced HBV reactivation in this study. HBV reactivation in patients infected with HBV genotype A was also not found in this study, which may be explained by the fact that this genotype occurs in only 1.7% of patients with chronic hepatitis B in Japan [31].

Because our study and other studies [23] have confirmed that HBV reactivation can be fatal, we need to emphasize greater testing of HBV markers, including antibody to hepatitis B core antigen, antibody to HBsAg, and HBV DNA levels before administration of chemotherapy, especially therapy containing rituximab. Patients with resolved HBV infection should be routinely monitored for liver function and HBV DNA levels, and antiviral therapy should be administered immediately when evidence of HBV reactivation is found.

In conclusion, HBV reactivation is found in 4% of newly HBsAg-positive patients with resolved HBV infection in Japan. One-fourth of cases of HBV reactivation develop into FHF, and mortality is extremely high. Because our study was unable to distinguish HBV reactivation from occult HBV infection and could not clarify whether antiviral therapy was effective, a prospective study is being planned to clarify the mechanism of HBV reactivation and the benefits of antiviral therapy.

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Table 3. Demographic and clinical characteristics of patients with hepatitis B virus (HBV) reactivation who did and did not experience fulminant hepatic failure (FHF).

Characteristic	Patients with HBV reactivation		
	Experienced FHF (n = 5)	Did not experience FHF (n = 18)	P
Age, median years (95% CI)	63 (47–64)	63 (39–78)	NS
Male sex	3 (60)	11 (61)	NS
Peak ALT level, median IU/L (95% CI)	907 (359–1823)	1016 (124–2524)	NS
Peak bilirubin level, median mg/dL (95% CI)	20.8 (10.2–45.7)	7.6 (0.3–24.9)	.094
Lowest albumin level, median g/dL (95% CI)	2.6 (2.1–3.0)	3.3 (2.2–3.6)	.015
Most prolonged PT%, median % (95% CI)	22.0 (8.7–32.3)	77.5 (18.0–101.8)	<.001
ALT level,[a] median IU/L (95% CI)	176 (83–1035)	266 (58–1690)	NS
Bilirubin level,[a] median mg/dL (95% CI)	0.7 (0.4–7.2)	0.7 (0.3–13.6)	NS
Albumin level,[a] median g/dL (95% CI)	3.4 (2.5–3.5)	3.9 (2.8–4.5)	.035
PT%,[a] median % (95% CI)	42.2 (16.4–46.4)	83.7 (38.7–123.5)	NS
HBV DNA level, median log copies/mL (95% CI)	7.6 (5.6 to >7.6)	7.5 (4.0 to >7.6)	NS
Genotype			
Bj	1 (20)	7/14 (50)	NS
C	4 (80)	7/14 (50)	NS
Mutation			
G1896A	4 (80)	5/12 (42)	NS
A1762T/G1764A	2 (40)	2/12 (17)	NS
Non-Hodgkin lymphoma	5 (100)	8 (44)	.046
Received a rituximab-containing treatment regimen	5 (100)	4 (22)	.004
Treatment			
Lamivudine	5 (100)	16 (89)	NS
IFN	1 (20)	4 (22)	NS
Liver-related death	5 (100)	1 (6)	<.001

NOTE. Data are no. (%) of patients, unless otherwise indicated. ALT, alanine aminotransferase; NS, not statistically significant; PT, prothrombin time.

[a] Laboratory data are from the start of lamivudine therapy.

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Techniques for the Early Detection of Drug Resistance

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Hepatitis B Core-Related Antigen Assay Is Useful for Monitoring the Antiviral Effects of Nucleoside Analogue Therapy

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Key Words

Drug resistance · HBV DNA · Hepatitis B core-related antigen · Lamivudine · Nucleoside analogue · Recurrence, hepatitis

Abstract

Objective: The clinical significance of the hepatitis B virus core-related antigen (HBcrAg) assay in monitoring the antiviral effects of lamivudine is reviewed. ***Methods:*** The HBcrAg assay simultaneously measured serum levels of hepatitis B core (HBc) and e (HBe) antigens using monoclonal antibodies which recognize common epitopes of these two denatured antigens. ***Results:*** Although serum HBcrAg levels correlated linearly with those of hepatitis B virus (HBV) DNA in natural course, the decrease in HBcrAg was significantly slower than in HBV DNA after initiation of lamivudine administration. We analyzed the clinical significance of HBV DNA and HBcrAg levels to predict the occurrence of lamivudine resistance. HBV DNA measurement may be useful to identify patients who are at high risk of developing lamivudine resistance, and HBcrAg measurement may help to detect patients who are at low risk of drug resistance. The measurement of HBcrAg was also found to be a useful prognosticator for reactivation of hepatitis after cessation of lamivudine administration. ***Conclusion:*** The HBcrAg assay is indeed useful for monitoring the antiviral effects of lamivudine, and we propose that it be adopted as a serum marker which reflects the amount of HBV covalently closed circular DNA in hepatocytes.

Introduction

Measurement of hepatitis B virus (HBV) DNA in serum is useful for monitoring the antiviral effects of nucleoside or nucleotide analogue therapy. However, a negative result for HBV DNA does not necessarily indicate a good therapeutic outcome since drug resistance may occur even if HBV DNA levels remain undetectable during therapy and reactivation of HBV replication may occur afterwards [1, 2]. Recently, a chemiluminescence enzyme immunoassay (CLEIA) was developed in our laboratory for the detection of hepatitis B core-related antigen (HBcrAg) [3, 4]. HBcrAg consists of HBV core and e antigens: both proteins are transcribed from the precore/core gene and their first 149 amino acids are identical [5–7]. HBcrAg CLEIA simultaneously measures serum levels of hepatitis B core and e antigens using monoclonal antibodies, which recognize common epitopes of these two denatured antigens. In the present report, we reviewed the clinical significance of the HBcrAg assay in monitoring the antiviral effects of lamivudine treatment.

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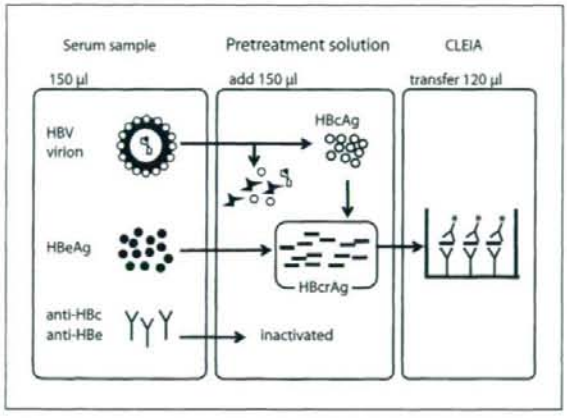

Fig. 1. Principles of the HBcrAg assay.

HBcrAg CLEIA

The principles of the HBcrAg assay are shown in figure 1. Briefly, 150 µl of serum sample are incubated with the same volume of pretreatment solution containing several detergents. Under these conditions, HBV virions are destroyed into denatured core proteins, e antigens are denatured and circulating antibodies to core and e antigens, which may interfere with the enzyme immunoassay (EIA), are inactivated. After incubation, 120 µl of pretreated specimen are subjected to the chemiluminescence EIA, and HBcrAg concentration is expressed as units per milliliter [3].

In serum samples not subjected to antiviral therapy, concentrations of HBcrAg and HBV DNA correlate linearly. The sensitivity of the HBcrAg assay for HBV viremia is better than that of the transcription-mediated amplification assay, which has a lower detection limit of 3.7 log copies/ml [8]. These findings indicate that the HBcrAg assay can measure viral load with high sensitivity [4].

Prediction of Lamivudine Resistance

Serum HBcrAg levels reflect viral load in the natural course because they correlate linearly with those of HBV DNA [4]. On the other hand, the characteristics of HBcrAg are somewhat different from HBV DNA in patients undergoing antiviral therapies, e.g. with lamivudine; specifically, the decrease in HBcrAg levels is significantly slower than that of HBV DNA after the initiation of lamivudine administration [9, 10].

We analyzed the clinical significance of HBV DNA and HBcrAg levels as a prognosticator of the occurrence of lamivudine resistance in a previous study [10]. Of the 81 patients enrolled, 27 (33%) showed lamivudine resistance during a median follow-up of 12 months. In patients without lamivudine resistance, HBV DNA decreased rapidly and remained below the detection limits in almost all patients. However, HBV DNA levels did not reach undetectable levels in the majority of patients with lamivudine resistance. Whereas HBcrAg levels decreased slowly to levels <4.7 log U/ml in most patients without lamivudine resistance, they did not decrease in cases with lamivudine resistance; HBcrAg levels remained >4.7 log U/ml until at least 6 months of treatment.

The cumulative occurrence of lamivudine resistance was then compared between both patient groups classified according to HBV DNA and HBcrAg levels after 6 months of therapy, with cutoff values for HBV DNA and HBcrAg set at 2.6 log copies/ml and 4.7 log U/ml, respectively. Lamivudine resistance at 2 years was as high as 80% in patients with HBV DNA ≥2.6 log copies/ml, but was about 30% in patients with levels <2.6 log copies/ml ($p < 0.001$). For HBcrAg, lamivudine resistance at 2 years was 50–60% in patients with HBcrAg levels ≥4.7 log U/ml, while no patients with levels below that developed lamivudine resistance ($p = 0.005$).

Taken together, our results suggest that measurement not only of HBV DNA, but also of HBcrAg, is useful to predict the occurrence of lamivudine resistance. HBV DNA measurement is valuable for identifying patients who are at high risk of developing lamivudine resistance, and measurement of HBcrAg is valuable for identifying those who are at low risk.

Reactivation of Hepatitis after Halting Lamivudine Administration

During lamivudine administration, the concentration of serum HBV DNA decreases and usually becomes undetectable even to high-sensitivity HBV DNA assays. However, this threshold may be an inadequate indicator for safely discontinuing lamivudine administration since active hepatitis often recurs in patients after treatment [11]. Therefore, we evaluated the clinical significance of the HBcrAg assay in predicting the likelihood of non-reactivation of hepatitis after discontinuing lamivudine administration for HBV treatment [9].

A total of 34 patients with chronic hepatitis B were enrolled, and lamivudine was administered for at least 6 months. Reactivation of hepatitis was defined as an elevation in ALT levels to >80 IU/l within 12 months of cessation.

Several factors just prior to lamivudine therapy were compared between patients with and without hepatitis reactivation after halting lamivudine administration, but did not significantly differ (e.g. age, gender, genotype, ALT, HBV DNA, HBcrAg or prevalence of HBeAg). Similarly, at the cessation of lamivudine therapy, several factors were compared, but the duration of lamivudine administration and the level of ALT did not differ between both groups and HBV DNA was below the detection limits in almost all patients. Thus, we can presume that HBV DNA did not differ between both groups. Interestingly, HBcrAg levels were significantly higher in patients with hepatitis reactivation than in those without (range 25–75%, 4.9; 4.7–5.9 vs. 3.2; <3.0–4.5 log U/ml, p = 0.009), indicating that measurement of HBcrAg is useful for predicting reactivation of hepatitis following lamivudine administration.

The ability of HBcrAg concentration to predict non-recurrence of hepatitis was analyzed using receiver-operating characteristic analysis, which showed a wide area under the curve of 0.764 in predicting non-reactivation of HBV. With the cutoff value set at 4.5 log U/ml, both specificity and sensitivity almost reached 80%. An even higher specificity of 0.9 can be obtained with an HBcrAg cutoff value of 4.0 log U/ml. In this case, the sensitivity would still be nearly 0.6.

Hypothesis

The replication process of HBV in hepatocytes is shown in figure 2. HBV is an enveloped DNA virus containing a relaxed circular DNA genome converted into a covalently closed circular DNA (cccDNA) episome in the nucleus of infected cells [7, 12–14]. These cccDNA molecules serve as the transcriptional templates for the production of viral RNAs that encode both viral structural and non-structural proteins. Reverse transcription of the viral pregenomic RNA and second-strand DNA synthesis occur in the cytoplasm within viral capsids formed by the HBV core protein. Because lamivudine, a nucleoside analogue, inhibits reverse transcription of pregenomic RNA, it directly suppresses the production of HBV virions [15, 16]. This explains why serum HBV DNA levels decrease rapidly after the initiation of lamivudine administration. On the other hand, the production of viral proteins is not suppressed by lamivudine because this process does not require reverse transcription. It has also been reported that the amount of HBV cccDNA, which serves as a template for mRNA, decreases quite slowly following commencement of nucleoside analogues [17–19]. Thus, it is reasonable that serum HBcrAg levels decrease much slower than HBV DNA levels after the initiation of lamivudine therapy.

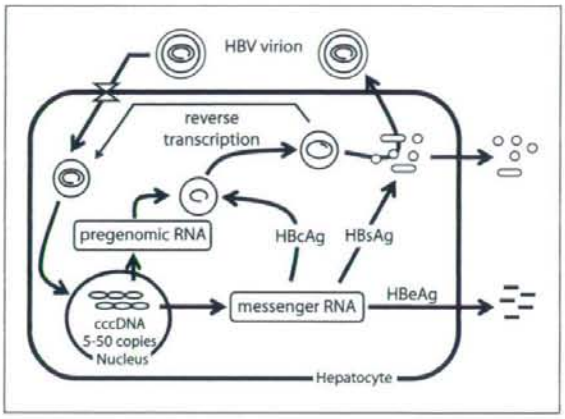

Fig. 2. Replication of HBV in hepatocytes.

Elevated levels of HBV cccDNA in hepatocytes have been associated with both more frequent occurrence of lamivudine resistance during lamivudine administration and more severe reactivation of hepatitis after discontinuation of lamivudine. Direct measurement of HBV cccDNA in hepatocytes is ideal for monitoring the antiviral effect of nucleotide analogues, but is not practical for clinical use because it requires a liver biopsy. Thus, a serum marker that reflects HBV cccDNA levels is needed. Our results suggest that serum HBcrAg levels reflect HBV cccDNA in hepatocytes more accurately than serum HBV DNA, which is also supported by the theoretical evidence described in the previous paragraph. We thus hypothesize that the HBcrAg assay can indeed be a representative serum marker which reflects the amount of HBV cccDNA in hepatocytes. Further studies are required to clarify the exact significance of the HBcrAg assay since a direct association between HBV cccDNA levels in hepatocytes and HBcrAg levels in serum remains to be shown.

Lamivudine has already been eliminated from first-line therapy in naïve chronic hepatitis B patients due to

the increased incidence of resistant mutations compared with newer antiviral agents, e.g. adefovir dipivoxil and entecavir [20]. The distinct characteristic of the HBcrAg assay under lamivudine therapy that differentiates it from other HBV DNA assays is that lamivudine suppresses the production of HBV virions by inhibiting reverse transcription of pregenomic RNA, but does not suppress the production of viral proteins, in which reverse transcription is unnecessary. Thus, it is possible that the HBcrAg assay may also be useful for patients undergoing entecavir or adefovir dipivoxil administration since the main mechanism of HBV replication suppression is similar. As a considerable number of patients who began lamivudine administration in the past are still taking this treatment now, the present study may be valuable for patients who consider changing therapy in the future, though further studies are required to determine whether the HBcrAg assay is indeed applicable to antiviral agents other than lamivudine.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

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CLINICAL STUDIES

Insulin resistance and hepatitis C virus: a case–control study of non-obese, non-alcoholic and non-steatotic hepatitis virus carriers with persistently normal serum aminotransferase

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Keywords
adiponectin – γ-glutamyltransferase – HCV core protein – insulin resistance – waist circumference

Abbreviations
ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet count ratio index; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; γ-GT, γ-glutamyltransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; HOMA, homoeostasis model assessment; hsCRP, high-sensitivity C-reactive protein; IR, insulin resistance; IRI, immunoreactive insulin; NASH, non-alcoholic steatohepatitis; PNALT, persistently normal serum alanine aminotransferase; TG, triglyceride; TNF, tumour necrosis factor; US, ultrasonography.

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Abstract

Background/Aims: Recent studies using transgenic mouse models have demonstrated that the presence of hepatitis C virus (HCV) singularly induces insulin resistance (IR). When evaluated in humans, the exclusion of other factors influencing IR, such as obesity, alcohol intake, hepatic inflammation and steatosis is needed, but only few studies have been performed to these ends. Therefore, we aimed at exploring the singular effects of HCV on glucose metabolism through analysis of HCV carriers with persistently normal serum aminotransferase. *Methods:* Non-obese, non-diabetic and non-alcoholic HCV carriers ($n = 30$) were enrolled with 30 hepatitis B virus carriers matched by age, gender, body mass index and waist-to-hip ratio. All patients maintained normal serum aminotransferase (<30 U/L), hyaluronic acid (<50 ng/ml) and platelet count ($>150 \times 10^3/\mu l$) for more than 5 years without additional treatments, and had no signs of steatosis. We then compared fasting plasma glucose, serum insulin and adiponectin, and homoeostasis model assessment of IR (HOMA-IR) and HOMA-β indices between the groups. *Results:* There were no significant differences in IR/secretion-associated markers or serum adiponectin. Multivariate analysis demonstrated that the presence of HCV was not an independent predictor of IR. HOMA-IR was strongly correlated with waist circumferences and serum γ-glutamyltransferase in HCV carriers, but not with serum aminotransferase, high-sensitivity C-reactive protein, hyaluronic acid or HCV core antigen. *Conclusions:* These results suggest that the presence of HCV alone does not affect IR. Coexistence of hepatitis, steatosis and/or fibrosis may be important to the pathogenesis of IR induced by chronic HCV infection.

There have been several epidemiological studies that have demonstrated a close association between chronic hepatitis C virus (HCV) infection and diabetes mellitus (1–3), and insulin resistance (IR) is the main feature of impaired glucose metabolism caused by HCV infection (4). Although the mechanism of IR has not been fully elucidated, increased triglyceride (TG) (5, 6) and/or iron accumulation (7–9) in the liver and advanced hepatic fibrosis (10–12) are thought to contribute to the phenomenon of IR. The

homoeostasis model assessment of IR (HOMA-IR) in the early stages of chronic hepatitis C patients is reported to be greater than that in healthy volunteers matched by age, gender, body mass index (BMI) and waist-to-hip ratio (13), suggesting the presence of a HCV-specific mechanism of IR independent of progression of hepatic fibrosis.

The possibility that HCV and/or the core protein itself can induce HCV-specific IR is based on several findings. Firstly, insulin receptor substrates-1 and -2, which are central molecules in the insulin signalling cascade, are downregulated in the livers of HCV core protein transgenic mice and in the core protein-transfected human hepatoma cell lines (14). Secondly, transgenic mice constitutively expressing core protein in livers exhibited marked hyperinsulinaemia and IR, which were ameliorated by inhibition of the tumour necrosis factor (TNF)-α pathway (15). Finally, HCV eradication by interferon therapy improved IR in patients with chronic hepatitis C (16). However, because factors such as obesity, hepatocyte injury, hepatic inflammation and steatosis have been shown to affect the onset of IR (17–19), they need to be excluded when the sole effect of HCV-specific IR in humans is discussed. As far as we know, there are very few studies that assess IR in chronically HCV-infected patients after careful adjustment for these factors. Hence, we sought to determine the contribution of HCV alone to the pathogenesis of IR by comparing the IR/secretion-related parameters between HCV and hepatitis B virus (HBV) carriers with persistently normal serum alanine aminotransferase (<30 U/L) (PNALT) (20). Before comparison, several other known factors that influence IR, such as obesity, alcohol intake (21), hepatic steatosis and fibrosis, were excluded, and both groups were carefully matched by age, gender, BMI and waist-to-hip ratio. IR was not found in non-obese, non-diabetic, non-alcoholic and non-steatotic HCV carriers with PNALT, implying that the presence of HCV *per se* cannot induce IR. In other words, our results suggest that various other hepatic abnormalities caused by HCV infection, including hepatitis, steatosis or fibrosis, are probably necessary for the pathogenesis of IR in chronically HCV-infected patients.

Patients and methods

Patients

Patient selection was carried out as shown in Figure 1. HCV and HBV carriers with PNALT were defined as patients who were positive for HCV-RNA and HBV surface antigen in sera, respectively, but who had normal serum alanine aminotransferase (ALT)

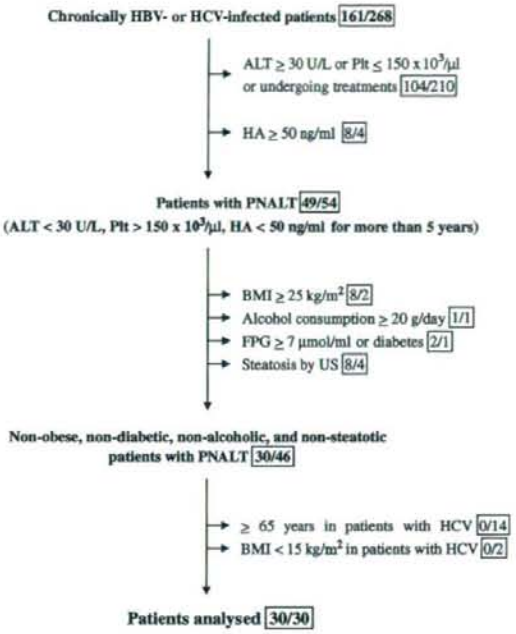

Fig. 1. Patient selection criteria. The left and right numbers in each box show the number of patients with chronic infection of HBV and HCV respectively. ALT, alanine aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HA, hyaluronic acid; HBV, hepatitis B virus; HCV, hepatitis C virus; Plt, platelet count; PNALT, persistently normal serum ALT; US, ultrasonography.

(<30 U/L), hyaluronic acid (<50 ng/ml) and platelet count (>150 × 10^3/μl) for more than 5 years without any treatments. Serum ALT and platelet count had been measured at least every 3 months, and serum hyaluronic acid had been measured every 6 months before the study. Patients who had previously been administered interferon injections and/or antiviral or hepatoprotective drugs were excluded. All HBV carriers were negative for hepatitis B e antigen and had viral loads <1000 copies/ml. To minimize any other factors affecting IR, additional criteria that have been strictly upheld for more than 5 years include : (i) BMI <25 kg/m^2, as calculated every 3 months; (ii) alcohol consumption <20 g/day; (iii) fasting plasma glucose (FPG) <7 μmol/ml, or taking no insulin or oral hypoglycaemic drugs; (iv) absence of steatosis, advanced fibrosis and cirrhosis, as detected by repeated abdominal ultrasonography (US) every 6 months; (v) absence of ongoing treatment with corticosteroids or any other medication known to affect glucose tolerance or insulin secretion and (vi) absence

of other concomitant diseases such as human immunodeficiency virus infection, hereditary haemochromatosis, pancreatitis, renal failure or neoplasia. Subsequently, both groups were carefully matched by age, gender, BMI and waist-to-hip ratio, and non-obese, non-diabetic, non-alcoholic and non-steatotic HCV ($n = 30$) and HBV ($n = 30$) carriers with PNALT were enrolled in this study (Fig. 1).

Informed consent, in writing, was obtained from all patients. Body height, weight and waist and hip circumferences were measured in the fasting state by hospital staff unaware of the patients' medical information. Any underlying diseases, medical interventions, past medical history and family history of diabetes were also recorded. Patients were considered hypertensive if their systolic/diastolic pressure was > 140/90 mmHg or if they were taking antihypertensive drugs. Patients were considered to have hyperlipidaemia if their fasting serum cholesterol and TG were ≥220 and 150 mg/dl, respectively, or if they were taking lipid-lowering drugs (5, 22). The presence of metabolic syndrome was judged according to the new definition released by the Japanese Committee for the Diagnostic Criteria of Metabolic Syndrome (23).

Laboratory examination

Venous blood samples were drawn from patients after an overnight fast. Serum insulin was determined by the radioimmunoassay method, and other data were measured by standard methods using a conventional automated analyser. HOMA-IR was calculated using the following equation: [FPG (μmol/ml) × immunoreactive insulin (IRI) (μU/ml)]/22.5. A HOMA-IR > 1.73 was considered indicative of the presence of IR, which was estimated using the *M*-value from the euglycaemic–hyperinsulinaemic clamp method in the Japanese population (24). HOMA-β, a parameter reflecting the insulin secretion ability of pancreatic β-cells, was calculated as follows: [360 × IRI (μU/ml)]/[FPG (μmol/ml)/0.0555–63]. Serum adiponectin was measured by means of an enzyme-linked immunosorbent assay kit (Otsuka Pharmaceutical Co. Ltd, Tokyo, Japan). Serum hyaluronic acid and high-sensitivity C-reactive protein (hsCRP) were examined by latex agglutination-turbidimetric immunoassay (Fujirebio Inc., Tokyo, Japan) and latex nephelometry (Dede Behring, Deerfield, IL, USA) respectively. The amount of HCV core antigen in serum was determined by a chemiluminescence enzyme immunoassay method (Eiken Chemical Co. Ltd, Tokyo, Japan). Serum aspartate aminotransferase-to-platelet count ratio index (APRI), a well-known indicator of hepatic fibrosis (25, 26), was calculated as follows: aspartate aminotransferase AST (U/L)/33 (upper limit of normal range of AST)/platelet count ($\times 10^3/\mu l$) × 100.

Imaging examination

Each patient underwent abdominal US (Hitachi model EUB-525 equipped with a 3.5 MHz convex-type transducer; Hitachi, Tokyo, Japan) in a fasting state. The presence of hepatic steatosis was assessed according to findings such as hepatorenal contrast, blurring of vascular walls and profound attenuation of the diaphragm (5, 27). The presence of advanced fibrosis or cirrhosis was evaluated by the presence of splenomegaly, hypertrophy of left or caudal lobes and surface irregularity (28). Images were evaluated and judged by an independent ultrasonographer uninformed about the clinical data of the patients.

Ethics

This study was carried out in accordance with the World Medical Association Helsinki Declaration and was approved by the hospital's human ethics committee.

Statistical analysis

Results are expressed as mean ± SD or median and range (in parenthesis). Statistical analyses were performed using SPSS software 11.5J for Windows (SPSS Inc., Chicago, IL, USA). Comparisons between the two groups were made using Fisher's exact probability test for categorical variables and the Student's *t* test for continuous variables. All *P*-values were based on a two-sided test of statistical significance. Correlation coefficients were calculated using Spearman's rank correlation analysis. A *P*-value of < 0.05 was considered statistically significant.

Results

Clinical features and biochemical parameters of hepatitis virus carriers with persistently normal serum alanine aminotransferase

The clinical features of both groups are shown in Table 1. The prevalence of hypertension and hyperlipidaemia was similar between the groups, and no participant had a family history of diabetes or fulfilled the Japanese criteria for metabolic syndrome. There were no significant differences in age, gender, BMI, waist-to-hip ratio, platelet count, hsCRP, serum AST or ALT, γ-glutamyltransferase (γ-GT), hyaluronic acid or APRI. Although it has been reported that serum cholesterol and ferritin are elevated in patients with chronic hepatitis C compared with those with chronic hepatitis B (29, 30), these factors were similar between

Table 1. Clinical features and biochemical parameters of the hepatitis virus carriers with persistently normal serum alanine aminotransferase

	HBV+	HCV+	*P*
n	30	30	
Age (years)	50 ± 9	53 ± 12	0.42
Gender (male/female)	7/23	8/22	1.00
Hypertension (*n*)	1 (3%)	1 (3%)	1.00
Hyperlipidaemia (*n*)	0 (0%)	1 (3%)	0.50
Metabolic syndrome (*n*)	0 (0%)	0 (0%)	–
BMI (kg/m^2)	21.2 ± 2.2	20.9 ± 2.6	0.71
Waist circumference (cm)	71.7 ± 8.8	71.8 ± 9.0	0.97
Hip circumference (cm)	90.0 ± 5.0	90.0 ± 6.3	0.99
Waist-to-hip ratio	0.80 ± 0.09	0.80 ± 0.08	0.99
Platelet (× 10^3/μl)	210 ± 34	223 ± 50	0.33
hsCRP (mg/dl)	0.036 ± 0.027	0.027 ± 0.023	0.26
Albumin (g/dl)	4.5 ± 0.2	4.4 ± 0.3	0.68
AST (U/L)	20 ± 3	22 ± 6	0.17
ALT (U/L)	17 ± 4	19 ± 6	0.14
γ-GT (U/L)	19 ± 11	24 ± 22	0.37
Choline esterase (U/L)	315 ± 106	282 ± 55	0.25
Total cholesterol (mg/dl)	202 ± 27	194 ± 40	0.46
TG (mg/dl)	71 ± 17	74 ± 36	0.77
HDL cholesterol (mg/dl)	69 ± 16	67 ± 15	0.64
Iron (μg/dl)	100 ± 38	103 ± 43	0.83
Transferrin saturation (%)	32 ± 13	31 ± 14	0.79
Ferritin (ng/ml)	84 ± 74	55 ± 50	0.13
Hyaluronic acid (ng/ml)	24 ± 14	25 ± 14	0.77
APRI	0.31 ± 0.07	0.33 ± 0.13	0.48
HCV core antigen (fmol/L)		10550 (95–25800)	

Qualitative data are expressed as a percentage and quantitative data are expressed as mean ± SD or median (range). The *P*-value for qualitative and quantitative data was calculated using Fisher's exact probability test and Student's *t* test respectively.

ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; γ-GT, γ-glutamyltransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; hsCRP, high-sensitivity C-reactive protein; HDL, high density lipoprotein; SD, standard deviation; TG, triglyceride.

the groups. HCV genotype was 1b in all patients, and overall HCV core antigen concentrations were variable.

Comparison of insulin resistance/secretion-related parameters between hepatitis C virus and hepatitis B virus carriers with persistently normal serum alanine aminotransferase

Next, serum insulin and adiponectin, as well as the parameters related to IR (HOMA-IR) and secretion (HOMA-β), were compared between HCV and HBV carriers. We found no significant differences in FPG (5.27 ± 0.72 vs. 5.33 ± 0.78 μmol/ml, $P = 0.83$), serum IRI (5.1 ± 2.8 vs. 7.9 ± 6.6 μU/ml, $P = 0.11$) or adiponectin (16.5 ± 6.3 vs. 13.9 ± 5.9 μg/ml, $P = 0.21$) (Table 2). HOMA-IR in HCV carriers was similar to that in HBV carriers (1.2 ± 0.8 vs. 1.8 ± 1.3, $P = 0.12$), and HOMA-β did not differ between the two groups (59 ± 31 vs. 123 ± 84, $P = 0.18$) as well. These results demonstrate that HCV-specific IR does not occur in non-obese, non-steatotic HCV carriers with PNALT.

Table 2. Comparison of parameters associated with insulin resistance/secretion between hepatitis B virus and hepatitis C virus carriers with persistently normal serum alanine aminotransferase

	HBV+	HCV+	*P*
n	30	30	
FPG (μmol/ml)	5.33 ± 0.78	5.27 ± 0.72	0.83
Glycohaemoglobin (%)	5.3 ± 0.3	5.2 ± 0.4	0.21
IRI (μU/ml)	7.9 ± 6.6	5.1 ± 2.8	0.11
HOMA-IR >1.73 (*n*)	7 (23%)	4 (13%)	0.51
HOMA-IR	1.8 ± 1.3	1.2 ± 0.8	0.12
HOMA-β	123 ± 84	59 ± 31	0.18
Adiponectin (μg/ml)	13.9 ± 5.9	16.5 ± 6.3	0.21

Qualitative data are expressed as a percentage and quantitative data are expressed as mean ± SD. The *P*-value for qualitative and quantitative data was calculated using Fisher's exact probability test and Student's *t* test respectively. HOMA-IR and HOMA-β were calculated according to the formulas described in 'Patients and methods'. A HOMA-IR of > 1.73 was judged positive for the presence of insulin resistance.

FPG, fasting plasma glucose; HBV, hepatitis B virus; HCV, hepatitis C virus; HOMA, homoeostasis model assessment; HOMA-IR, homoeostasis model assessment of insulin resistance; IRI, immunoreactive insulin; SD, standard deviation.

Multivariate analysis

Multivariate analysis was performed to investigate the contribution of HCV infection to IR. There were no independent predictors of IR, including the presence of HCV.

Correlation between clinical parameters and insulin resistance/secretion markers in hepatitis C virus carriers with persistently normal serum alanine aminotransferase

To explore the clinical indicators associated with IR/secretion in the HCV carriers with PNALT, correlations between several clinical parameters with HOMA-IR, serum IRI/adiponectin and HOMA-β were analysed. HOMA-IR was strongly correlated with waist circumference ($r = 0.580$, $P = 0.006$), serum γ-GT ($r = 0.554$, $P = 0.004$) and TG ($r = 0.529$, $P = 0.007$) (Table 3). HOMA-IR was also associated with hip circumference ($r = 0.496$, $P = 0.022$), but this was weaker than that of the aforementioned indicators. Interestingly, HOMA-IR did not correlate with serum adiponectin ($r = -0.303$, $P = 0.170$), which was inversely correlated with waist

Table 3. Correlations between immunoreactive insulin/homoeostasis model assessment of insulin resistance/adiponectin/homoeostasis model assessment-β and clinical parameters in hepatitis C virus carriers with persistently normal serum alanine aminotransferase

	IRI	HOMA-IR	Adiponectin	HOMA-β
Waist circumference	0.600**	0.580**	-0.567**	NS
Hip circumference	0.525*	0.496*	NS	0.452*
Waist-to-hip ratio	NS	NS	-0.700**	NS
γ-GT	0.546**	0.554**	-0.631**	NS
TG	0.508**	0.529**	NS	0.410*
HDL-cholesterol	NS	NS	0.473*	NS

Correlation coefficients were calculated by Spearman's rank correlation analysis.

*$P < 0.05$.

**$P < 0.01$.

γ-GT, γ-glutamyltransferase; HDL, high density lipoprotein; HOMA, homoeostasis model assessment; HOMA-IR, homoeostasis model assessment of insulin resistance; IRI, immunoreactive insulin; NS, not significant; TG, triglyceride.

circumference ($r = -0.567$, $P = 0.007$), waist-to-hip ratio ($r = -0.700$, $P < 0.001$) and γ-GT ($r = -0.631$, $P = 0.002$), and positively correlated with high-density lipoprotein–cholesterol ($r = 0.473$, $P = 0.026$). On the other hand, there were no significant correlations between HOMA-IR or serum adiponectin with serum AST, ALT, hsCRP, platelet count, hyaluronic acid, APRI, HCV core antigen, ferritin or transferrin saturation ratio. The HOMA-β was weakly associated with hip circumference ($r = 0.452$, $P = 0.040$) and serum TG ($r = 0.410$, $P = 0.042$). No similar correlations were found in asymptomatic HBV carriers, demonstrating the existence of a positive and strong association between HOMA-IR/serum adiponectin, waist circumference and serum γ-GT and TG in chronically HCV-infected patients.

Discussion

In the current study, there were no significant differences in HOMA-IR and serum adiponectin between non-obese, non-diabetic, non-alcoholic and non-steatotic HCV and HBV carriers with PNALT. According to multivariate analysis, the presence of HCV was not an independent predictor of IR. HOMA-IR in the HCV carriers was strongly associated with waist circumference and serum γ-GT and TG, but not with the indicators of obesity (BMI), hepatocyte injury (serum AST or ALT), hepatic fibrosis (platelet count, serum hyaluronic acid or APRI), iron accumulation (serum ferritin or transferrin saturation ratio), systemic inflammation (serum hsCRP) or amount of HCV core protein. These results support the premise that the presence of HCV alone cannot induce IR. To our knowledge, this is the first study to evaluate HOMA-IR and serum adiponectin in non-obese, non-diabetic HCV carriers with PNALT and compare them with HBV carriers.

We defined the presence of IR as a HOMA-IR of > 1.73. Although considerably lower than that in Hispanic and Caucasian populations, this cutoff value is consistent with the results of a previous study using young, lean, healthy individuals (31) that showed a significantly lower insulin sensitivity index in Asian groups compared with other ethnic groups.

Several lines of evidence have shown that the presence of obesity, alcohol consumption and hepatic steatosis all contribute to the onset of IR (5, 6, 17, 18, 21). To exclude these factors as much as possible, we first selected non-obese hepatitis virus carriers devoid of a history of habitual alcohol intake and hepatic steatosis and closely matched by BMI and waist-to-hip ratio. Our results revealed that waist circumference, an important anthropometric predictor of visceral fat accumulation (32), was significantly correlated with HOMA-IR and inversely associated with serum adiponectin in HCV carriers. Surprisingly, BMI was not found to strongly affect HOMA-IR in our cohort. Several studies have documented a close relationship between visceral fat accumulation and IR in healthy volunteers (33, 34), and the results of this study support such a strong contribution to IR development in chronically HCV-infected patients as well.

We judged the presence of hepatic steatosis using abdominal US. Although US is a safe, non-invasive and accurate method of detecting moderate-to-severe steatosis, its diagnostic accuracy declines sharply in cases of mild steatosis (liver fat $< 25\%$). Indeed, clear differentiation between non-alcoholic steatohepatitis (NASH) with mild steatosis or cryptogenic chronic hepatitis is sometimes difficult by such imaging modalities. We previously reported that in Japanese patients with persistent ALT elevation, despite no detection of steatosis by US, obesity, hyperferritinaemia and high HOMA-IR are predictors of NASH with mild steatosis (5). The close relationship between IR and hepatic steatosis has also been documented elsewhere in the Asian population (31). In this study, patients with a BMI of more than 25 kg/m^2 were excluded, and most patients demonstrated normal serum ferritin and HOMA-IR. Thus, the possibility that patients with mild steatosis were included is presumably low.

Because advanced fibrosis may also lead to hyperinsulinaemia probably because of decreased insulin clearance capacity (11, 12), we also limited our patients to those having no or mild fibrosis, as estimated