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Materials and methods

Patient selection

The study protocol was approved by the Institutional Ethics Committee of Ogaki Municipal Hospital in January 2011, and was in compliance with the Declaration of Helsinki. Written informed consent for the use of stored serum samples for the study was obtained from all patients.

Between 1998 and 2008, 1220 consecutive HBsAg-positive patients, who visited the Department of Gastroenterology and Hepatology at Ogaki Municipal Hospital, were prospectively enrolled in our HCC surveillance program. Of these, 785 patients met the following inclusion criteria: HBsAg positive for more than 6 months, no evidence of HCV co-infection, exclusion of other causes of chronic liver disease (alcohol consumption >80 g/day, hepatotoxic drugs, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, or Wilson's disease), follow-up duration of greater than 3 years, no evidence of HCC for at least 1 year from the start of the follow-up period, receiving no interferon treatment, and receiving NA therapy for more than 1 year before the detection of HCC (Fig. 1). In patients on NA therapy, the date of NA therapy initiation was considered the starting point of the follow-up period.

Of these 785 patients, 148 received NA therapy (NA group) and 637 patients did not receive NA therapy (non-NA group) during the follow-up period. To reduce the confounding effects of covariates, we used propensity scores to match NA patients to unique non-NA patients. Six covariates including age, sex, HBV DNA concentration, hepatitis B e antigen (HBeAg), platelet count, and alanine aminotransferase (ALT) activity were taken into account at the start of follow-up. We computed the propensity score by using logistic regression with the independent variable including age (⩽40 years or >40 years), sex (female or male), HBV DNA concentration (⩽5.0 log copies/ml or >5.0 log copies/ml), HBeAg (negative or positive), platelet count (>150 × 10^3/m^3 or ⩽150 × 10^3/m^3), and ALT activity (⩽40 IU/ml or >40 IU/ml), as shown in previous reported cut-off values according to the indication for NA therapy [16–19]. This model yielded a *c* statistic of 0.85 (95% confidence interval [CI], 0.82–0.88), indicating very good ability of the propensity score model to predict treatment status. We sought to match each patient who received NA therapy to a patient who did not receive NA therapy, having a propensity by using greedy 5–1 digit matching [20]. Once this threshold was exceeded, a patient with NA therapy was excluded. This score ranged from 0.09198 to 0.98967 and, in effect, represented the probability that a patient would be receiving NA. We were able to match 117 patients with NA therapy to 117 unique patients without NA therapy. The follow-up period ended on 31 December, 2011 or the date when HCC occurrence was identified.

Surveillance and diagnosis

All patients were followed up at our hospital at least every 6 months. During each follow-up examination, platelet count, ALT, gamma glutamyl transpeptidase (gamma-GTP), total bilirubin, alkaline phosphatase (ALP), albumin, and alpha-fetoprotein (AFP) levels were measured. We used commercially available kits to test blood samples for HBsAg, HBeAg, and anti-HBe (Abbott Japan Co., Ltd., Tokyo, Japan). Before November 2007, the serum HBV DNA concentration was monitored by a polymerase chain reaction assay (COBAS Amplicor HBV monitor test, Roche Diagnostics K. K., Tokyo, Japan) with a lower detection limit of approximately 2.6 log copies/ml, and after December 2007, it was monitored with another polymerase chain reaction assay (COBAS AmpliPrep-COBAS TaqMan HBV Test, Roche Diagnostics K. K.), with a lower detection limit of approximately 2.1 log copies/ml. HBV genotyping was performed as described previously [21]. Serum levels of HBV core-related antigen (HBcrAg) were measured using a chemiluminescence enzyme immunoassay (CLEIA) as described previously [22,23]. Precore nucleotide 1896 and basal core promoter (BCP) dinucleotide 1762/1764 were determined using the line probe assay (INNO-LiPA HBV PreCore assay; Innogenetics NV) [24,25]. The probes were designed to determine the nucleotides at position 1896 (G *vs.* A) in the precore region and positions 1762 (A *vs.* T) and 1764 (G *vs.* A and G *vs.* T) in the BCP region. A line probe assay was used to identify any emergence of YMDD mutations (INNO-LiPA HBV DR assay; Innogenetics NV).

Platelet count, ALT, gamma-GTP, total bilirubin, ALP, albumin, AFP, and HBV DNA values were expressed as average integration values [26,27] after the start of follow-up.

According to the Clinical Practice Guidelines for Hepatocellular Carcinoma in Japan [28], we performed ultrasound (US) and monitoring of 3 biomarkers (AFP, *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein [AFP-L3], and des-gamma-carboxy prothrombin [DCP]) every 3–4 months, and dynamic magnetic resonance imaging (MRI) every 12 months, for patients with cirrhosis under surveillance. For patients with chronic hepatitis, we performed US and monitoring of the 3 biomarkers every 6 months. Histological examinations were performed in 91 out of 234 patients. Among them, cirrhosis was diagnosed in 32 patients. In the remaining 143 patients, the diagnosis of cirrhosis was made according to typical US findings, e.g., superficial nodularity, a coarse parenchymal echo pattern, and signs of portal hypertension (splenomegaly >120 mm, dilated portal vein diameter >12 mm, patent collateral veins, or ascites) [29–31]. Patients who did not satisfy these criteria were classified as having chronic hepatitis. One hundred and forty-two patients were diagnosed with chronic hepatitis and 92 patients with cirrhosis. For diagnostic confirmation of HCC, patients underwent dynamic MRI. A histological diagnosis of HCC was made in 28 patients (surgical specimen, 23 patients; US-guided needle biopsy specimen, 5 patients). The remaining 29 patients were diagnosed with HCC based on typical dynamic MRI findings, including hypervascularity in the arterial phase with washout in the portal venous or delayed phase [32].

Treatments

In the NA group, 117 patients received NA therapy including 18 patients with lamivudine, 28 patients with lamivudine and adefovir dipivoxil, and 71 patients with entecavir. The indications for NA therapy followed the guidelines of the American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver (EASL), or the Asian Pacific Association for the Study of the Liver (APASL) [33–35]. In contrast, of the 117 patients not on NA therapy, 104 did not receive treatment before NA was not yet approved in Japan and the remaining 13 patients declined NA therapy.

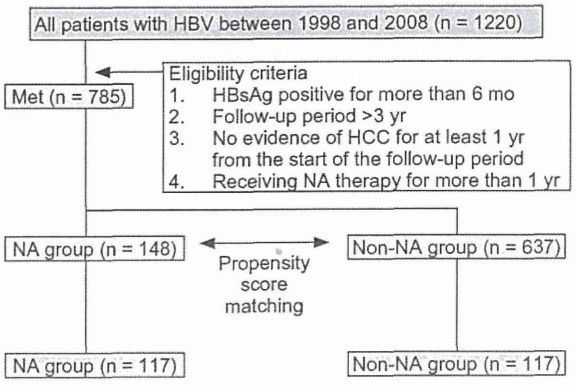

Fig. 1. Flowchart of the patient selection process.

Statistical analysis

Continuous variables are expressed as medians (range). The Mann–Whitney *U* test was used for continuous variables, and the Chi-square test with Yates' correction or Fisher's exact test was used for categorical variables. Actuarial analysis of the cumulative incidence of hepatocarcinogenesis was performed using the Kaplan–Meier method, and differences were tested with the log-rank test. The Cox proportional hazards model and the forward selection method were used to estimate the relative risk of HCC associated with age (⩽40 years or >40 years), sex (female or male), treatment (NA or no NA), HBsAg (⩽3.0 log IU/ml or >3.0 log IU/ml), HBV DNA level (⩽5.0 log copies/ml or >5.0 log copies/ml), HBeAg (negative or positive), precore region (wild type or mutant), BCP (wild type or mutant type), HBcrAg (⩽3.0 logU/ml or >3.0 logU/ml), platelet count (>150 × 10^3/m^3 or ⩽150 × 10^3/m^3), ALT (⩽40 IU/ml or >40 IU/ml), total bilirubin, gamma-GTP, ALP, albumin, and AFP (⩽10 ng/ml or >10 ng/ml) for univariate and multivariate analyses. We used the minimum or maximum of the reference values at our institution as cut-off values for total bilirubin, gamma-GTP, ALP, and albumin. We conducted a sensitivity analysis to determine the magnitude of an unmeasured confounder [36].

We considered *p* values of 0.05 or less to be significant. Statistical analysis was performed with SPSS, version 18.0 for Windows (International Business Machines Corporation, Tokyo, Japan).

Table 1. Baseline characteristics of all patients.

	NA group (n = 148)	Non-NA group (n = 637)	*p* value	Standardized difference in %
Age (yr)	53 (26-81)	48 (4-85)	<0.0001	40.6
Sex (female/male)	60/88	285/352	0.5378	6.1
Genotype (A/B/C/D/F/n.d.)	2/5/137/0/1/2	24/60/389/2/0/162	<0.0001	37.6
HBsAg (log_{10} IU/ml)	3.5 (-0.1-5.5)	3.3 (-1.3-7.9)	<0.0001	53.8
HBV DNA (log_{10} copies/ml)	7.0 (2.6-9.6)	3.8 (2.3-9.9)	<0.0001	99.9
HBeAg (±)	76/72	151/486	<0.0001	62.8
Precore region (W/M/n.d.)	30/109/9	88/381/168	0.4652	0.0
BCP (W/M/n.d.)	33/123/10	135/279/205	0.0074	27.3
HBcrAg (log_{10} U/ml)	5.9 (2.9-7.0)	3.0 (2.9-7.0)	<0.0001	96.7
Platelet count (x10^3/m^3)	150 (32-388)	188 (37-503)	<0.0001	-59.7
ALT (IU/ml)	65 (7-1088)	26 (5-3410)	<0.0001	44.1
AFP (ng/ml)	3.9 (0.8-3363)	2.9 (0.8-3686)	0.0062	-6.2
Cirrhosis (presence/absence)	62/86	91/546	<0.0001	59.1
Child-Pugh classification (A/B)	132/16	618/19	0.0002	32.7
Follow-up duration (yr)	12.8 (3.1-19.6)	13.7 (3.1-20.0)	0.1565	-16.9
Administration period (yr)	6.5 (1.5-11.0)	-	-	-
Propensity score	0.58093 (0.09198-0.98686)	0.95253 (0.12913-0.98967)	<0.0001	-132.3

NA, nucleos(t)ide analogue; n.d., not done; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; W, wild type; M, mutant type; BCP, basal core promoter; HBcrAg, hepatitis B core-related antigen; ALT, alanine aminotransferase; AFP, alpha-fetoprotein; Child-Pugh classification, reference no [50], Standardized difference in%; $100(X_{NA} - X_{non\text{-}NA})/([s^2_{NA} + s^2_{non\text{-}NA}]/2)^{1/2}$, where for each covariate X_{NA} and $X_{non\text{-}NA}$ are the sample means in NA and non-NA groups, respectively, and s^2_{NA} and $s^2_{non\text{-}NA}$ are the corresponding sample variances.

Results

Patient characteristics

Table 1 shows baseline characteristics of all 785 patients before propensity matching. There were significant differences in age, HBV genotype, HBsAg, HBV DNA concentration, presence of HBeAg, BCP mutations, HBcrAg, platelet counts, ALT level, AFP level, presence of cirrhosis, and Child-Pugh classification. The baseline characteristics of the 234 study patients after propensity matching are summarized in Table 2. There are no significant differences in age, sex, HBV genotype, HBsAg, HBV DNA concentration, presence of HBeAg, precore region mutations, BCP mutations, platelet counts, ALT concentration, Child-Pugh classification, and follow-up duration. HBcrAg concentration was significantly higher in the NA group than in the non-NA group. NA was administered a median of 6.1 years (range: 1.5–10.7 years).

Factors associated with the incidence of hepatocarcinogenesis

Factors associated with the incidence of HCC as determined by the Cox proportional hazard models and the forward selection method were analyzed in all 785 patients. High age (hazard ratio, 6.43 [95% CI, 2.71–15.26], p <0.001), male sex (3.43 [1.67–7.02], p = 0.002), NA treatment (0.28 [0.21–0.85], p = 0.017), BCP mutation (19.96 [2.27–141.90], p = 0.03), high HBcrAg levels (8.21 [3.40–19.85], p <0.001), and high AFP levels (2.49 [1.43–4.34], p = 0.001) were significantly associated with the incidence of HCC.

HCC developed in 57 of 234 patients (24.4%) during follow-up after propensity matching. The 5-year, 7-year, and 10-year cumulative incidences of HCC were 9.6%, 20.4%, and 33.4%, respectively. The 5-year, 7-year, and 10-year cumulative incidences of HCC were 2.7%, 3.3%, and 3.3%, respectively, in patients on NA therapy (n = 117) and 11.3%, 26.0%, and 40.0% in patients not on NA therapy (n = 117). Hepatocarcinogenesis occurred at significantly higher rates in the non-NA group (p = 0.0094, Fig. 2). The 5-year, 7-year, and 10-year cumulative incidences of HCC were 0.0%, 0.0%, and 0.0%, respectively, in patients with wild type BCP (n = 38) and 11.0%, 25.2%, and 41.9% in patients with mutant BCP (n = 112; p = 0.0006, Fig. 3). Factors associated with the incidence of HCC as determined by the Cox proportional hazard models and the forward selection method are listed in Table 3. Higher age (hazard ratio, 4.36 [95% CI, 1.33–14.29], p = 0.015), NA treatment (0.28 [0.13–0.62], p = 0.002), BCP mutation (12.74 [1.74–93.11], p = 0.012), high HBcrAg levels (2.77 [1.07–7.17], p = 0.036), and high gamma-GTP levels (2.76 [1.49–5.12], p = 0.001) were significantly associated with the incidence of HCC. In addition, 2 patients died due to hepatic failure during the follow-up period in the non-NA group.

The sensitivity analysis found that the observed relationship between NA treatment and HCC incidence could be diminished by the unmeasured confounder that the high prevalence of the unmeasured confounder is greater in the non-NA group than in the NA group. For example, suppose a binary unmeasured confounder that increased the hazard of HCC incidence (hazard ratio, 1.50) was present in 40% of those who were treated with NA and 80% of those who were not treated with NA. Then, the study's result would become less extreme and would no longer be statistically significant (hazard ratio under sensitivity analysis, 0.48 [95% CI, 0.22–1.05]).

Follow-up data of various parameters in patients on or not on NA therapy

For this analysis, we used the average integration value during the follow-up period (Table 4). ALT, gamma-GTP, ALP, AFP, and

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Table 2. Baseline characteristics of patients on NA therapy and propensity-matched controls.

	NA group (n = 117)	Non-NA group (n = 117)	*p* value	Standardized difference in %
Age (yr)	52 (27-77)	52 (21-77)	0.9223	1.7
Sex (female/male)	44/73	45/72	0.8929	6.1
Genotype (A/B/C/n.d.)	1/4/109/3	4/7/85/21	0.1232	26.8
HBsAg (log_{10} IU/ml)	3.6 (0.9-5.5)	3.6 (0.9-7.9)	0.1440	29.9
HBV DNA (log_{10} copies/ml)	6.7 (2.6-9.6)	6.5 (2.3-9.6)	0.1273	20.5
HBeAg (±)	57/60	58/59	0.8960	2.0
Precore region (W/M/n.d.)	22/87/8	16/75/26	0.6399	5.1
BCP (W/M/n.d.)	22/88/7	17/70/30	0.9359	0.0
HBcrAg (log_{10} U/ml)	5.9 (2.9-7.0)	4.9 (2.9-7.0)	0.0022	41.2
Platelet count ($x10^3/m^3$)	143 (32-262)	146 (37-396)	0.6340	-12.1
ALT (IU/ml)	68 (7-1088)	55 (9-3410)	0.0977	1.9
AFP (ng/ml)	2.8 (0.8-402)	3.9 (0.8-1010)	0.3118	-13.5
Cirrhosis (presence/absence)	48/69	44/73	0.6882	6.1
Child-Pugh classification (A/B)	108/9	104/13	0.5024	3.1
Follow-up duration (yr)	12.3 (3.1-19.4)	11.6 (3.1-18.3)	0.7346	-4.5
Administration period (yr)	6.1 (1.5-10.7)	-	-	-
Propensity score	0.65895 (0.11449-0.96977)	0.65895 (0.12913-0.96989)	0.9931	0.0

NA, nucleos(t)ide analogue; n.d., not done; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; W, wild type; M, mutant type; BCP, basal core promoter; HBcrAg, hepatitis B core-related antigen; ALT, alanine aminotransferase; AFP, alpha-fetoprotein; Child-Pugh classification, reference no [50], Standardized difference in%; $100(X_{NA} - X_{non\text{-}NA})/([S^2_{NA} + S^2_{non\text{-}NA}]/2)^{1/2}$, where for each covariate X_{NA} and $X_{non\text{-}NA}$ are the sample means in NA and non-NA groups, respectively, and S^2_{NA} and $S^2_{non\text{-}NA}$ are the corresponding sample variances.

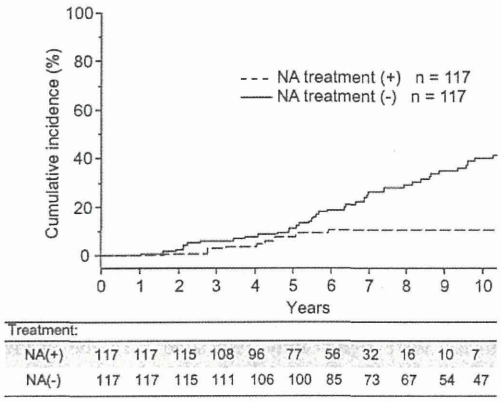

Fig. 2. Incidence of hepatocellular carcinoma (HCC) according to nucleos(t)ide analogue (NA) treatment status. The NA group had a significantly higher rate of progression to HCC than the non-NA group ($p = 0.0094$).

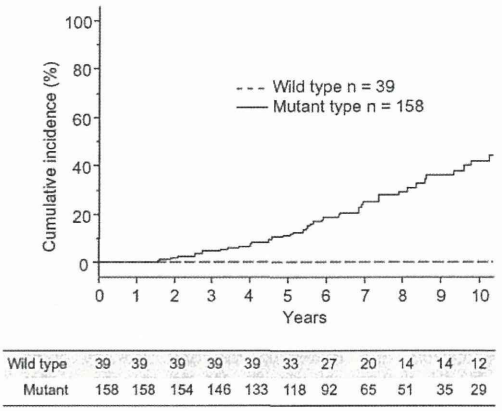

Fig. 3. Incidence of hepatocellular carcinoma (HCC) according to basal core promoter (BCP) mutations. Patients with mutant-type BCP had a significantly higher rate of progression to HCC than those with wild type BCP ($p = 0.0006$).

HBV DNA levels were significantly lower in patients on NA therapy than in patients not on NA therapy. In contrast, platelet counts and albumin levels were significantly higher in patients on NA therapy than in patients not on NA therapy.

Discussion

Our study shows that long-term NA maintenance therapy is associated with the suppression of progression to HCC. Liaw *et al.* reported that lamivudine decreased the risk of HCC in cirrhotic patients [7]. However, it is unclear whether the observed decreased risk of HCC with NA therapy was due to the short observation period in their study. It is very difficult to prove the preventive effect of NA on the development of HCC, because randomized control studies are not ethically possible. In this study, patients on NA therapy were compared to propensity score-matched untreated controls. In these control patients, NA therapy had not yet been approved or was not routinely used for chronic hepatitis B at the time, or was declined by the patient. As opposed to the entire population, these propensity-matched patients were well matched to patients on NA; significant differences included higher HBcrAg levels in the NA group.

Large community-based studies have confirmed that advanced age, male sex, HBeAg positivity, low platelet count,

Table 3. Factors associated with progression to hepatocellular carcinoma among propensity-matched patients (Cox proportional hazard model).

		Adjusted hazard ratio (95% CI)	*p* value
Age (yr)	≤40	1	0.015
	>40	4.36 (1.33-14.29)	
Treatment	no NA	1	0.002
	NA	0.28 (0.13-0.62)	
BCP	wild-type	1	0.012
	mutant-type	12.74 (1.74-93.11)	
HBcrAg (log_{10} U/ml)	≤3.0	1	0.036
	>3.0	2.77 (1.07-7.17)	
γ-GTP (IU/L)	≤56	1	0.001
	>56	2.76 (1.49-5.12)	

NA, nucleos(t)ide analogue; BCP, basal core promoter; HBcrAg, hepatitis B core-related antigen; γ-GTP, gamma glutamyl transpeptidase.

higher ALT levels, elevated AFP levels, and presence of cirrhosis are factors associated with the development of cirrhosis and HCC [17,18]. Platelet count is a useful surrogate marker for the diagnosis of cirrhosis [37]. All subjects were not histologically diagnosed in this study. Therefore, we selected platelet count as a marker of hepatic fibrosis instead of cirrhosis. An elevated ALT level indicates the presence of active disease, and persistently elevated AFP levels are a reflection of an enhanced regenerative state in the liver [16]. In the REVEAL study, a high HBV DNA load was associated with an increased rate of HCC development [17]. A direct correlation was observed between baseline HBV DNA levels and the incidence of HCC, independent of serum ALT concentration. In a model that integrated baseline and follow-up HBV DNA levels, the cumulative incidence of HCC ranged from 1.3% in patients with undetectable levels of HBV DNA to 14.9% in patients with HBV DNA levels greater than or equal to 10^6 copies/ml. Therefore, we have selected factors, such as age, sex, HBeAg serostatus, HBV DNA concentration, platelet count, and ALT for propensity matching.

Although the exact mechanisms of hepatocarcinogenesis by HBV remain unclear, two mechanisms have been proposed [38,39]. One mechanism involves chronic necroinflammation of hepatocytes, cellular injury, and hepatocyte regeneration [40]. The other mechanism involves the direct carcinogenicity of HBV through chromosomal integration [41]. Complete and sustained viral suppression by NA might block both pathways and prevent the development of HCC. It is well known that the rate of HCC is significantly higher in patients with virological breakthrough or no response. In our study, when virological or biochemical breakthrough was observed and the YMDD mutation was detected in patients on lamivudine, adefovir dipivoxil was immediately added. In patients with cirrhosis, especially in the decompensated stage, sustained viral response on NA therapy was not necessarily associated with a preventative effect against the development of HCC, even though the incidence was lower than in a group not on NA [14]. It is not surprising that viral suppression decreased but did not eliminate the risk of HCC, because HBV DNA may have already integrated into the host genome before the initiation of therapy and may have resulted in genomic alternations, chromosomal instability, or both [42,43].

It is reported that patients with HBV genotype C infection have higher HBV DNA levels, higher frequency of pre-S deletions, higher prevalence of BCP T1762/A1764 mutations, and significantly higher chances of developing HCC [16,44–46]. In our study, T1762/A1764 mutations were observed in 158 (80.2%) out of 197 patients and were associated with a higher risk of developing HCC (adjusted hazard ratio, 12.740 [95% CI 1.743–93.108]), independent of NA therapy. However, the BCP T1762/A1764 mutations were detected in HCC patients from Asia and Africa, where HBV genotype C infection is predominant [16].

HBcrAg is a new HBV marker that reflects HBV load and corresponds to HBV DNA levels [21]. HBcrAg is comprised of HBV core antigen (HBcAg) and HBeAg; both are products of the precore/core gene and share the first 149 amino acids of HBcAg. The HBcrAg assay measures HBcAg and HBeAg simultaneously by using monoclonal antibodies that recognize both denatured HBcAg and HBeAg [47]. Serum HBcrAg concentration is well correlated with intrahepatic levels of covalently closed circular DNA (cccDNA) [48]. It is reported that HBcrAg is a useful marker for guiding cessation of NA therapy and evaluation of disease activity [21,49]. In our study, elevated serum HBcrAg concentration was associated with a higher risk of developing HCC (adjusted hazard ratio, 2.767 [95% CI 1.067–7.172]). This is the first report demonstrating a relationship between HBcrAg and HCC.

The present study has several limitations. The retrospective design might have introduced an unintended bias. The propensity matching method was adopted to reduce the confounding effects of covariates. Characteristics of patients who did or did not receive NA therapy were similar except for HBcrAg concentration.

Table 4. Average integration values of various parameters in patients who did or did not receive NA therapy.

	NA group (n = 117)	Non-NA group (n = 117)	*p* value
Platelet count (x10^3/m^3)	17.0 (3.3-37.2)	14.8 (3.3-296)	0.0060
ALT (IU/ml)	28.2 (8.5-88.9)	39.1 (12.2-737.5)	<0.0001
γ-GTP (IU/L)	27.0 (10.9-267.6)	36.2 (9.5-269.7)	0.0427
Total bilirubin (mg/dl)	0.7 (0.3-2.0)	0.7 (0.3-2.6)	0.1554
ALP (IU/L)	242.7 (113.5-1028.8)	265.2 (140.5-1247.6)	0.0127
Albumin (g/dl)	4.4 (3.0-5.0)	4.0 (2.4-4.8)	<0.0001
Alpha-fetoprotein (ng/ml)	2.2 (0.8-106.0)	4.5 (0.9-723.8)	<0.0001
HBV DNA (log_{10} copies/ml)	2.5 (2.1-8.9)	4.6 (2.1-9.3)	<0.0001

NA, nucleos(t)ide analogue; ALT, alanine aminotransferase; γ-GTP, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; HBV, hepatitis B virus.

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However, the non-NA group included many historical cases when NA therapy was not yet available. In addition, the HBV DNA assay used between 1998 and 2007 was not the most sensitive one.

In conclusion, NA therapy reduces the risk of HCC compared with untreated controls. Higher serum HBcrAg levels and BCP mutations are associated with development of HCC, independent of NA therapy.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Clinicopathological features of liver injury in patients with type 2 diabetes mellitus and comparative study of histologically proven nonalcoholic fatty liver diseases with or without type 2 diabetes mellitus

Toshihide Shima · Hirofumi Uto · Kohjiro Ueki · Toshinari Takamura · Yutaka Kohgo · Sumio Kawata · Kohichiroh Yasui · Hyohun Park · Naoto Nakamura · Tatsuaki Nakatou · Nobuyoshi Tanaka · Atsushi Umemura · Masayuki Mizuno · Junko Tanaka · Takeshi Okanoue

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Abstract
Background The Japan Society of Diabetes Mellitus reported that the leading cause of death in patients with diabetes mellitus (DM) was chronic liver disease; however, there are limited studies investigating the cause of liver injury in these patients. Our study aimed to clarify the clinicopathological features of liver injury and the characteristics of nonalcoholic fatty liver disease (NAFLD) in DM patients.
Methods In total, 5,642 DM patients and 365 histologically proven NAFLD patients were enrolled. Clinical and laboratory parameters and liver biopsy results were, respectively, recorded and analyzed for the two sets of patients.
Results Positivity rates for Hepatitis B surface antigens (HBsAg) and anti-hepatitis C virus antibodies (anti-HCV Ab) were 1.7 and 5.1 %, respectively. The proportion of drinkers consuming 20–59 g and ≥60 g alcohol daily was 14.9 and 4.3 %, respectively. The percentage of DM patients with elevated serum alanine aminotransferase (ALT) levels (≥31 IU/L) was 28.6 %. Alcohol consumption had no significant effect on serum ALT levels. Seventy-two percent of HBsAg-positive patients were serum hepatitis B virus (HBV)-DNA negative, whereas 10 % exhibited high levels of the same (>4.0 log copies/ml).

T. Shima · A. Umemura · M. Mizuno · T. Okanoue (✉)
Department of Gastroenterology and Hepatology, Saiseikai Suita Hospital, Suita, Japan
e-mail: okanoue@suita.saiseikai.or.jp

H. Uto
Digestive and Life-style Related Disease, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

K. Ueki
Diabetes and Metabolic Diseases, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

T. Takamura
Disease Control and Homeostasis, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan

Y. Kohgo
Gastroenterology and Hematology/Oncology, Department of Medicine, Asahikawa Medical College, Asahikawa, Japan

S. Kawata
Gastroenterology, Yamagata University Faculty of Medicine, Yamagata, Japan

K. Yasui
Molecular Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, Kyoto, Japan

H. Park
Diabetes and Metabolic Diseases, Saiseikai Suita Hospital, Suita, Japan

N. Nakamura
Endocrinology and Metabolism, Kyoto Prefectural University of Medicine, Kyoto, Japan

T. Nakatou
Diabetes Center, Okayama Saiseikai General Hospital, Okayama, Japan

N. Tanaka
Gastroenterology, Fukui-ken Saiseikai Hospital, Fukui, Japan

J. Tanaka
Department of Epidemiology, Infectious Disease Control and Prevention, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan

Thirty-eight percent of anti-HCV Ab-positive patients were serum HCV-RNA negative. Among the NAFLD patients, the frequencies of NASH and advanced stage NASH were significantly higher in male DM patients than in male patients without DM.

Conclusions Although HBsAg- and anti-HCV Ab-positivity rates were high in our Japanese DM patients, a majority of liver injuries could be associated with NAFLD/nonalcoholic steatohepatitis.

Keywords Nonalcoholic fatty liver disease · Nonalcoholic steatohepatitis · Diabetes mellitus · Hepatitis virus carrier · Alcoholic liver disease · Nationwide study

Abbreviations

HCC	Hepatocellular carcinoma
NAFLD	Nonalcoholic fatty liver disease
DM	Diabetes mellitus
NASH	Nonalcoholic steatohepatitis
HBV	Hepatitis B virus
HCV	Hepatitis C virus
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
GGT	Gamma glutamyl transpeptidase
FPG	Fasting plasma glucose
HOMA-IR	The homeostasis model assessment of insulin resistance index
HBsAg	Hepatitis B surface antigen
anti-HBc Ab	Anti-hepatitis B core antibody
anti-HCV Ab	Anti-hepatitis C virus antibody
HBV-DNA	Hepatitis B virus-deoxyribonucleic acid
HCV-RNA	Hepatitis C virus-ribonucleic acid
OR	Odds ratio
CI	Confidence interval

Introduction

As per the International Diabetes Federation, the number of diabetes mellitus (DM) sufferers rose to 366 million in 2011, representing 8.3 % of the global adult population, which is increasing in every country [1]. Worldwide, hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer mortality [2]. HCC largely occurs in patients with chronic liver disease. Persistent hepatitis C virus (HCV) or hepatitis B virus (HBV) infections are the main causes of HCC; however, non-HCV- and non-HBV-associated HCC cases are increasing in Japan [3].

In 2007, the Japan Society of DM reported that the most frequent cause of death among 18,385 DM patients who died in hospitals during 1991–2000 was malignancy (34.1 %), followed by ischemic heart disease (10.2 %) and cerebrovascular disease (9.8 %) [4]. Among the malignancies, HCC showed the highest frequency (8.6 %), followed by lung (5.3 %), pancreatic (4.8 %), and gastric cancer (3.5 %). Furthermore, the frequency of deaths caused by liver cirrhosis was 4.7 %, and in total, 13.3 % DM patients died of liver diseases. The cancer death rate in that study was quite different from that reported in the general Japanese population, in which lung (5.7 %), gastric (4.7 %), and colon (2.5 %) cancer occur with high frequencies [5]. Moreover, the death rate from liver diseases (13.3 %) was three times higher than that in the general Japanese population (HCC 3.2 %, liver cirrhosis 1.5 %, total 4.7 %) [6]. However, the incidences of HBV and HCV infection and the details of alcohol intake were not analyzed in that report.

The Japan Nonalcoholic Steatohepatitis (NASH) Study Group was founded in 2007 to investigate the cause of death in DM patients, the genetic factors in nonalcoholic fatty liver disease (NAFLD) patients, and the background of NASH-HCC patients [7]. This study focused on clarifying the cause of liver injury in Japanese DM patients and investigating the histological distribution of NAFLD in patients with and without DM.

Patients and methods

Patients

In total, 5,642 DM patients (3,238 males, 2,404 females) who visited nine DM clinics belonging to the Japan NASH Study Group (Saiseikai Suita Hospital; Kagoshima University Graduate School of Medical and Dental Sciences; Graduate School of Medicine, The University of Tokyo; Kanazawa University Graduate School of Medical Science; Department of Medicine, Asahikawa Medical College; Yamagata University Faculty of Medicine; Kyoto Prefectural University of Medicine; Okayama Saiseikai General Hospital; Fukui-ken Saiseikai Hospital) between January 2008 and December 2009 were enrolled in this observational study.

Three hundred and sixty-five NAFLD patients (182 males, 183 females) who visited Saiseikai Suita Hospital were enrolled in the histopathological study.

The study protocol was approved by the Human Ethics Committee of each participating hospital. Informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

Clinical and laboratory assessment

Demographic parameters, including age, sex, height, weight, and body mass index (BMI), and comorbidities, including alcohol consumption, hypertension, and dyslipidemia, were

recorded for all subjects in addition to the treatment administered for DM and the frequency of HCC occurrence. Clinical laboratory tests were conducted to measure aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), albumin, total cholesterol, triglyceride (TG), ferritin, uric acid, hemoglobin A1c, fasting plasma glucose (FPG), and insulin levels. The homeostasis model assessment of insulin resistance (HOMA-IR) index; platelet (PLT) count; and hyaluronic acid, type IV collagen 7S, hepatitis B surface antigen (HBsAg), anti-hepatitis B core antibody (anti-HBc Ab), anti-HCV antibody (anti-HCV Ab), HBV-DNA, and HCV-RNA levels were also measured.

Blood samples were procured in the morning after overnight fasting. HOMA-IR was only calculated for subjects with FPG <140 mg/dL. HBV-DNA levels were measured by PCR (Amplicor HBV-DNA kit, Roche Diagnostics) or real-time PCR (TaqMan HBV-DNA kit, Roche Diagnostics) for HBsAg-positive, whereas HCV-RNA levels were measured by PCR (Amplicor HCV-RNA kit, version 2.0, Roche Diagnostics) or real-time PCR (TaqMan HCV-RNA kit, Roche Diagnostics) for anti-HCV Ab-positive patients.

Histopathological examination

In total, 365 patients (177 non-DM and 188 DM) at Saiseikai Suita Hospital fulfilled the criteria for NAFLD, namely serum HBsAg and anti-HCV Ab negativity, no alcohol consumption, and the absence of autoimmune liver diseases or hereditary liver injury. These patients underwent an ultrasound-guided liver biopsy using a 16G needle.

Specimens were fixed in formalin, embedded in paraffin, and subjected to hematoxylin–eosin, Masson trichrome, and Perl's iron staining. Histological features of samples were interpreted according to a method described by Matteoni et al [8]. NASH stage was classified according to Brunt's classification [9].

Statistical analysis

All statistical analyses were performed using SPSS for Windows (SPSS Japan Inc.). Data were summarized by frequency for categorical variables and mean ± standard deviation (SD) for continuous variables. The Chi-square test was used to determine the differences between categorical variables. Student's t test was used to compare means of continuous valuables with equal variance, and the Mann–Whitney U test was used for non-normally distributed variables. The Cochran–Armitage test was used to study the trend of continuous variables. Forward stepwise logistic regression analysis was used to identify independent variables related to elevated serum ALT (≥31 IU/L) levels. A p value of <0.05, obtained by a two-tailed test, was considered statistically significant.

Since there is no official report on the HBV and HCV carrier rate in the general Japanese population, we utilized blood donor data for comparison with our patients [10].

Results

Baseline characteristics

The mean age and BMI of male and female DM patients was 62.2 and 64.8 years and 24.5 and 24.7 kg/m^2, respectively (Table 1). Hypertension and dyslipidemia occurred in 51.0 and 63.3 % of DM patients, respectively. Respective DM treatment types in DM patients were as follows: no medication, 20.5 %; oral drugs, 47.7 %; insulin, 28.9 %; and oral drugs and insulin, 2.8 % (Table 2).

Mean ALT level was significantly higher in males (30.6 IU/L) than in females (Table 1). Abnormal serum ALT levels (≥31 IU/L) were found in 28.6 % of DM patients (males 32.8 %, females 23.0 %). When the healthy upper limit of abnormal serum ALT level in females was defined as 20 IU/L according to Prati et al.'s [11] criteria, the frequency of abnormal ALT (≥21 IU/L) levels in females was 43 %. The mean PLT count was 20.8 × 10^4/μL in males and 21.9 × 10^4/μL in females. Mean values of other clinical laboratory tests are shown in Table 1.

Prevalence of HBV and HCV infection and drinking and their effects on liver function tests

HBsAg positivity was detected in 1.7 % of DM patients (M 1.8 %, F 1.6 %) (Table 2); this was significantly higher than that (total 0.9 %, M 1.0 %, F 0.7 %) detected in 1.7 million blood donors aged >40 years ($p < 0.001$). For both sexes, the HBsAg detection rate was significantly higher in DM patients than in blood donors in the 50- to 59- and 60- to 69-year age groups ($p < 0.05$) (Fig. 1). There were no significant differences in serum AST, ALT, and GGT levels between HBsAg-positive and HBsAg-negative DM patients of both sexes.

Seventy-two percent of HBsAg-positive patients (M 69 %, F 79 %) demonstrated HBV-DNA negativity (<2.6 log copies/ml) (Table 3). Of the HBsAg-positive patients, only 10 % showed high serum HBV-DNA levels (≥4.0 log copies/ml); these could be HBV infection-induced liver injury cases. Mean values of age, serum ALT level, and PLT counts in HBV-DNA-negative HBV carriers were 63.6 years, 25.3 IU/L, and 20.5 × 10^4/μL, respectively. HBV-DNA-negative HBV carriers were older and exhibited lower ALT levels and higher PLT counts; however, the differences were not significant.

Table 1 Backgrounds of diabetes mellitus patients (1)

Characteristic	Total subjects		Males		Females		
	n	M ± SD	*n*	M ± SD	*n*	M ± SD	*p*
Age (years)	5,510	63.3 ± 12.7	3,164	62.2 ± 12.5	2,346	64.8 ± 12.9	<0.001
BMI (kg/m^2)	5,173	24.6 ± 4.7	2,987	24.5 ± 4.2	2,186	24.7 ± 5.2	0.629
Aspartate aminotransferase (IU/L)	5,568	26.4 ± 17.2	3,188	27.1 ± 18.0	2,380	25.5 ± 15.9	<0.001
Alanine aminotransferase (IU/L)	5,569	28.2 ± 24.5	3,190	30.6 ± 26.9	2,379	24.9 ± 20.5	<0.001
GGT (IU/L)	5,476	48.3 ± 72.5	3,131	59.6 ± 86.7	2,345	33.1 ± 42.9	<0.001
Albumin (g/dL)	5,031	4.2 ± 0.4	2,869	4.2 ± 0.5	2,162	4.1 ± 0.4	<0.001
Platelet (×10^4/μL)	5,419	21.3 ± 6.1	3,112	20.8 ± 6.0	2,307	21.9 ± 6.1	<0.001
Fasting plasma glucose (FPG; mg/dL)	5,123	152.7 ± 61.7	2,945	156.0 ± 63.9	2,178	148.3 ± 58.2	<0.001
HbA1c (%)	5,479	7.2 ± 1.7	3,143	7.2 ± 1.7	2,336	7.2 ± 1.6	0.744
HOMA-IR (FPG <140)	1,005	2.55 ± 2.60	570	2.51 ± 2.59	435	2.61 ± 2.60	0.209
Total cholesterol (mg/dL)	5,260	195.1 ± 39.5	3,016	191.6 ± 40.0	2,244	199.6 ± 38.5	<0.001
Triglycerides (mg/dL)	5,443	136.3 ± 102.7	3,119	145.1 ± 111.9	2,324	124.5 ± 87.5	<0.001
Hyaluronic acid (ng/mL)	559	74.5 ± 98.6	319	59.3 ± 73.0	240	94.6 ± 122.1	<0.001
Type 4 collagen 7S (ng/mL)	474	4.9 ± 2.0	269	4.8 ± 2.0	205	4.9 ± 1.9	0.544
Ferritin (ng/mL)	1,838	142.0 ± 157.0	1,084	171.9 ± 174.9	754	99.1 ± 114.1	<0.001
Uric acid (mg/dL)	3,645	5.4 ± 1.5	2,043	5.7 ± 1.4	1,602	4.9 ± 1.4	<0.001

Results are shown as mean ± SD

GGT gamma glutamyl transpeptidase, *HOMA-IR* homeostasis model assessment of insulin resistance

Table 2 Backgrounds of diabetes mellitus patients (2)

Characteristic	Total subjects		Males		Females		
	n	Positive (%)	*n*	Positive (%)	*n*	Positive (%)	*p*
Hepatitis B surface antigen	4,867	83 (1.7 %)	2,796	50 (1.8 %)	2,071	33 (1.6 %)	0.604
Anti-hepatitis B core antibody	3,211	958 (29.8 %)	1,824	572 (31.4 %)	1,387	386 (27.8 %)	0.030
Anti-hepatitis C virus antibody	4,877	247 (5.1 %)	2,812	144 (5.1 %)	2,065	103 (5.0 %)	0.834
Alcohol	4,443		2,554		1,889		<0.001
<20 g/day		3589 (80.8 %)		1769 (69.3 %)		1820 (96.3 %)	
20–59 g/day		661 (14.9 %)		609 (23.8 %)		52 (2.8 %)	
≥60 g/day		193 (4.3 %)		176 (6.9 %)		17 (0.9 %)	
Hypertension	4,936	2515 (51.0 %)	2,841	1419 (49.9 %)	2,095	1096 (52.3 %)	0.100
Dyslipidemia	5,423	3434 (63.3 %)	3,091	1882 (60.9 %)	2,332	1552 (66.6 %)	<0.001
Diabetes mellitus (intervention)	5,227		3,013		2,214		0.002
None		1072 (20.5 %)		629 (20.9 %)		443 (20.0 %)	
Oral drugs		2495 (47.7 %)		1489 (49.4 %)		1006 (45.4 %)	
Insulin		1513 (28.9 %)		810 (26.9 %)		703 (31.8 %)	
Oral drugs + insulin		147 (2.8 %)		85 (2.8 %)		62 (2.8 %)	
Hepatocellular carcinoma	4,700	67 (1.4 %)	2,696	48 (1.8 %)	2,004	19 (0.9 %)	0.017

Anti-HCV Ab positivity was detected in 5.1 % (M 5.1 %, F 5.0 %) of DM patients; this rate was significantly higher than that (total 1.0 %, M 1.1 %, F 1.0 %) in blood donors of every age group of both sexes ($p < 0.001$), except for females aged 50–59 years (Table 2; Fig. 2). ALT and GGT levels were significantly higher in male anti-HCV Ab-positive patients than in their negative counterparts ($p < 0.001, p < 0.05$) (Fig. 2). For both sexes,

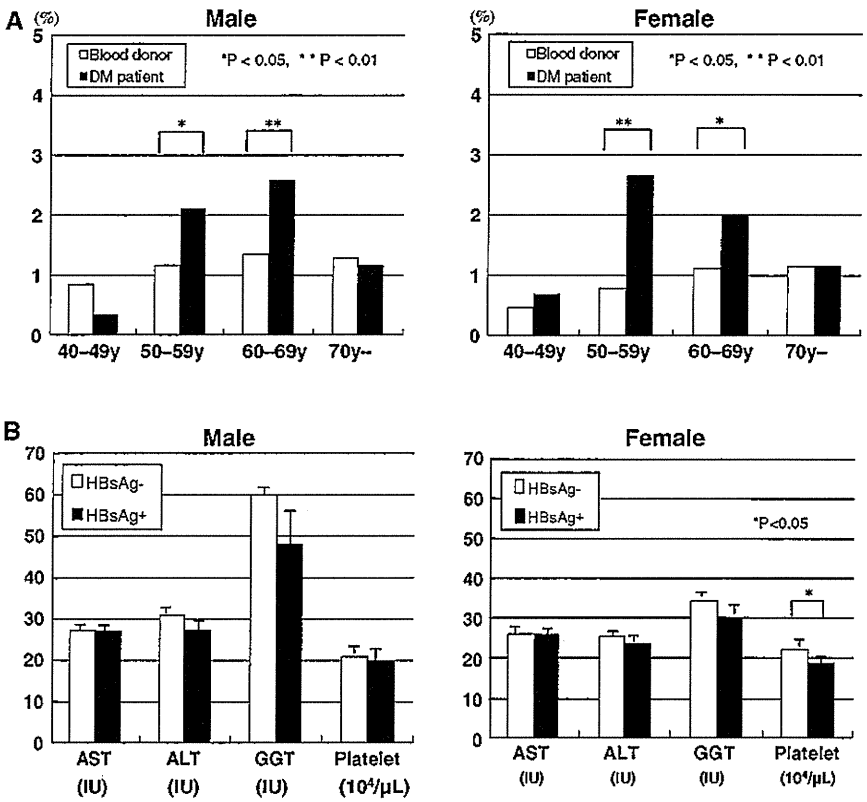


Fig. 1 Prevalence of HBV infection and the effect of HBV infection on laboratory tests in DM patients. a Prevalence of HBV infection in blood donors and DM patients. b The effect of HBV infection on laboratory tests in DM patients. There were no significant differences in serum AST, ALT, and GGT levels between HBsAg-positive and HBsAg-negative DM patients of both sexes. *Error bars* SD

Table 3 Serum HBVDNA and HCVRNA levels, age, serum ALT level, and platelet (PLT) counts in HBsAg-positive patients and anti-HCV Ab-positive patients

	% (*n*)	Mean age (years)	Mean ALT levels (IU/L)	Mean PLT count($\times 10^4/\mu L$)
Serum HBV-DNA[a]				
Negative (<2.6 log copy/ml)	72 (29)	63.6	25.3	20.5
Positive (=2.6 log copy/ml)	28 (11)	55.6	28.0	18.5
=2.6<4.0	18 (7)	61.9	26.6	18.9
=4.0	10 (4)	50.0	30.5	17.8
Serum HCV-RNA[b]				
Negative (<2.7 log IU/ml)	38 (57)	67.2	28.2	17.7
Positive (=2.7 log IU/ml)	62 (91)	67.4	51.7	15.3
=2.7<5.0	3 (4)	65.1	28.0	16.4
=5.0	59 (87)	67.5	52.7	15.2

[a] Results are presented as either frequency or mean in 40 HBsAg-positive patients

[b] Results are presented as either frequency or mean in 148 anti-HCV Ab-positive patients

the PLT count was significantly lower in anti-HCV Ab-positive DM patients than in their negative counterparts ($p < 0.001$).

Thirty-eight percent of anti-HCV Ab-positive patients (M 36 %, F 42 %) demonstrated HCV-RNA negativity (Table 3), and 96 % of HCV-RNA-positive patients exhibited high serum HCV-RNA levels (≥5.0 log IU/ml). Serum ALT levels in anti-HCV Ab-positive patients with HCV-RNA positivity and those with HCV-RNA negativity were 51.7 ± 39.7 and 28.2 ± 18.1 IU/L, respectively, whereas those in anti-HCV Ab-negative patients were 27.7 ± 22.8 IU/L. Serum ALT levels were significantly higher in HCV-RNA-positive patients than in HCV-RNA-negative patients ($p < 0.001$).

The proportion of DM patients consuming >60 g and 20–59 g alcohol daily was 4.3 % (M 6.9 %, F 0.9 %) and

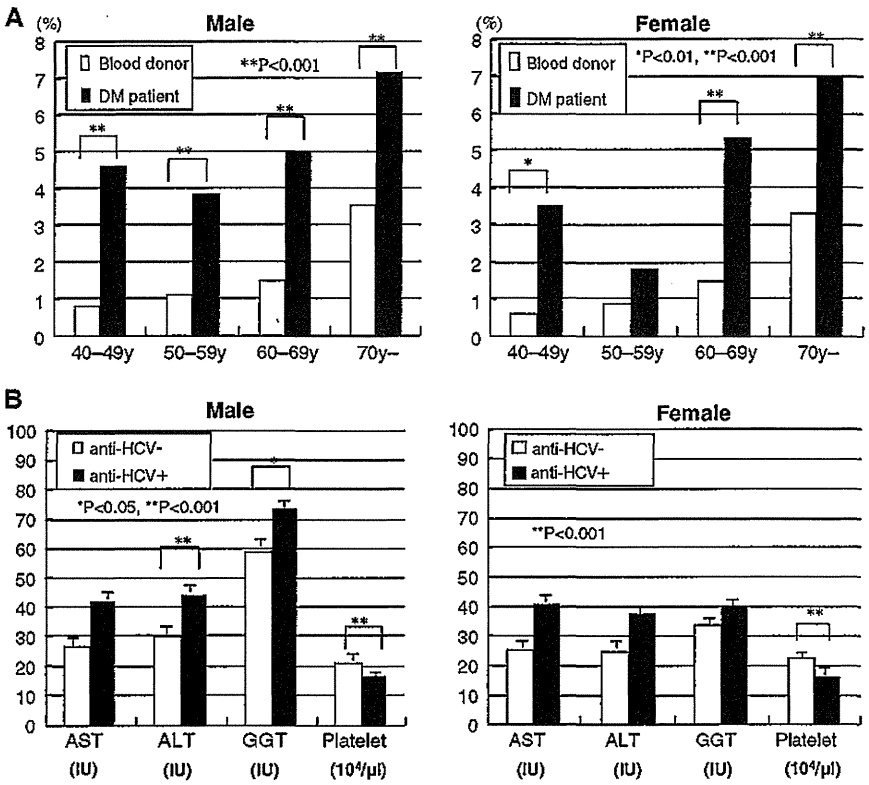


Fig. 2 Prevalence of HCV infection and the effect of HCV infection on laboratory tests in DM patients. a Prevalence of HCV infection in blood donors and DM patients. b The effect of HCV infection on laboratory tests in DM patients. *GGT* gamma glutamyl transpeptidase. ALT and GGT levels were significantly higher in male anti-HCV Ab-positive patients than in their negative counterparts. *Error bars* SD

14.9 % (M 23.8 %, F 2.8 %), respectively (Table 2). The highest percentage of drinkers were males in the 60- to 69-year age group and females in the <40-year age group. Male drinkers consuming >60 g alcohol daily had significantly higher serum AST and GGT levels compared with nondrinkers (patients consuming <20 g of daily alcohol intake) ($p < 0.001$). Serum ALT levels in drinkers consuming >60 g alcohol daily were comparable with those in nondrinkers. Drinkers of both sexes consuming 20–59 g alcohol daily had significantly higher serum GGT levels ($p < 0.001$) (Fig. 3).

Factors related to serum ALT levels

With increasing age in both sexes, the number of DM patients with elevated serum ALT levels and high BMI decreased, whereas those with decreased PLT counts increased. The number of DM patients with elevated serum ALT levels increased with increasing BMI in both sexes (Fig. 4).

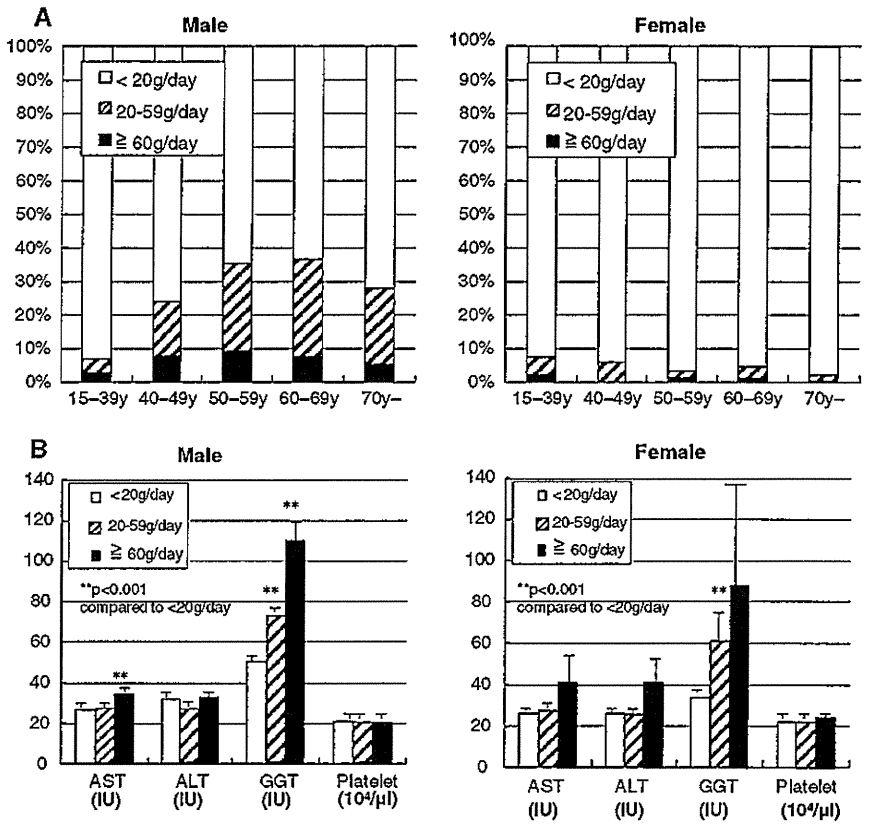
A forward stepwise logistic regression model yielding odds ratios (ORs) and 95 % confidence intervals (CIs) was used to analyze the factors related to elevated serum ALT levels. The model included BMI, age, drinking status, HBsAg status, anti-HCV Ab status, PLT count, hypertension status, and dyslipidemia status as independent variables. The odds ratio shown indicates the change in odds for one SD increase in each variable.

Multivariate analysis showed that age (M: OR 0.674, CI 0.613–0.741; $p < 0.001$; F: OR 0.767, CI 0.683–0.861; $p < 0.001$), PLT count (M: OR 0.806, CI 0.732–0.886; $p < 0.001$, F: OR 0.714, CI 0.632–0.808, $p < 0.001$), anti-HCV Ab status (M: OR 1.321, CI 1.218–1.433; $p < 0.001$; F: OR 1.232, CI 1.117–1.359; $p < 0.001$), and BMI (M: OR 1.509, CI 1.374–1.657; $p < 0.001$; F: OR 1.487, CI 1.330–1.663; $p < 0.001$) were significantly associated with elevated serum ALT levels (Table 4).

For both sexes, AST and ALT levels were similar in drinkers consuming 20–59 g alcohol daily and those consuming <20 g alcohol daily (Fig. 3). After eliminating HBV-positive patients and/or HCV carriers and heavy drinkers consuming >60 g alcohol daily, the number of male, female, and total DM patients with elevated serum ALT levels were 33.4, 23.3, and 28.3 %, respectively. These values were comparable with those in all DM patients, including those with hepatitis and/or those consuming alcohol (M 32.8 %, F 23.0 %, total 28.6 %).

Liver histology in DM patients

The median age of histologically proven, DM- ($n = 87$) and non-DM-associated ($n = 95$) male NAFLD patients



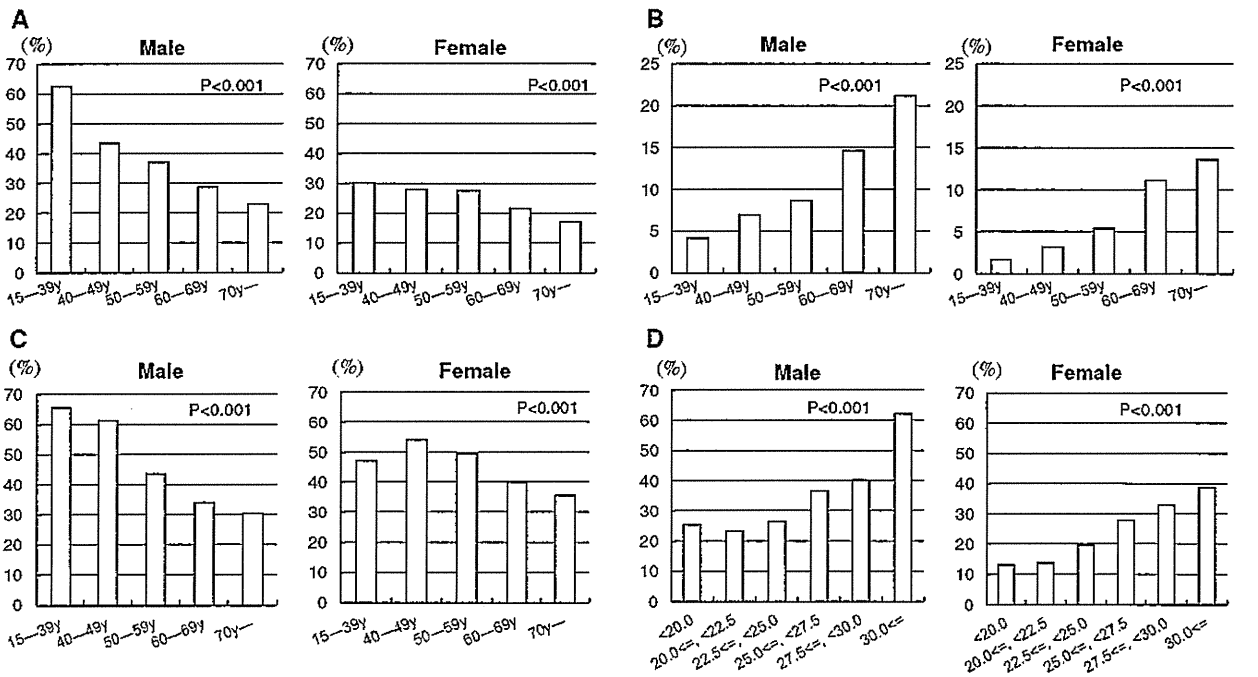


Fig. 3 Drinking habits and the effect of alcohol consumption on laboratory tests in DM patients. **a** Drinking habits in individual age. **b** The effect of alcohol consumption on laboratory tests in DM patients. *GGT* gamma glutamyl transpeptidase. Serum ALT levels in drinkers consuming >60 g alcohol daily were comparable with those in nondrinkers. *Error bars* SD

Fig. 4 Influence of age on the ratio of patients with elevated serum ALT level, decreased PLT count and abnormal BMI, and the relationship between BMI and the ratio of patients with elevated serum ALT level. **a** The ratio of patients with elevated serum ALT level (≥31 IU/L). **b** The ratio of patients with decreased PLT count (<15 × 10^4/μL). **c** The ratio of patients with abnormal BMI (≥25). **d** The relationship between BMI and the ratio of patients with elevated serum ALT level (≥31 IU/L)

Table 4 Multivariate analysis to identify independent variables related to elevated serum ALT level (≥31 IU/L)

	Regression coefficient	Standard error	Odds ratio	95 % confidence interval	*p*
Males					
Age	−0.394	0.048	0.674	0.613–0.741	<0.001
Platelet	−0.216	0.049	0.806	0.732–0.886	<0.001
Anti-hepatitis C virus	0.278	0.042	1.321	1.218–1.433	<0.001
Body mass index	0.411	0.048	1.509	1.374–1.657	<0.001
Females					
Age	−0.265	0.059	0.767	0.683–0.861	<0.001
Platelet	−0.336	0.063	0.714	0.632–0.808	<0.001
Anti-hepatitis C	0.208	0.050	1.232	1.117–1.359	<0.001
Body mass index	0.397	0.057	1.487	1.330–1.663	<0.001

was 60 and 45 years, respectively; in corresponding females, the median age was 66 ($n = 101$) and 61 years ($n = 82$), respectively. No significant difference was noted in BMI between DM and non-DM NAFLD patients (M 26.0 and 27.5 kg/m^2, respectively; F 26.0 and 27.0 kg/m^2, respectively). Male NAFLD patients without DM were significantly younger than those with DM ($p < 0.001$).

NAFLD patients were classified according to Matteoni's classification. Type 1, 2, 3, and 4 cases were 14 (16 %), 14 (16 %), 4 (5 %), and 55 (63 %), respectively, among male DM patients and 28 (29 %), 23 (24 %), 11 (12 %), and 33 (35 %), respectively, among male non-DM patients. Type 1, 2, 3, and 4 cases were 16 (16 %), 8 (8 %), 4 (4 %), and 73 (72 %), respectively, among female DM patients and 9 (11 %), 9 (11 %), 7 (9 %), and 57 (69 %), respectively, among female non-DM patients (Fig. 5). The frequency of Type 4 NASH was significantly higher in male DM patients than in male non-DM patients ($p < 0.001$).The rate of Type 4 NASH was high in both female DM and non-DM patients.

In total, 244 (M 103, F 141) NASH patients were classified according to Brunt's classification. The number of patients with stage 0 (Matteoni Type 3), 1, 2, 3, and 4 were 2 (3 %), 27 (46 %), 10 (17 %), 17 (29 %), and 3 (5 %), respectively, among male DM patients and 10 (23 %), 16 (36 %), 8 (18 %), 9 (21 %), and 1 (2 %), respectively, among male non-DM patients. Stage 0, 1, 2, 3, and 4 cases were 4 (5 %), 25 (33 %), 24 (31 %), 13 (17 %), and 11 (14 %), respectively, among female DM patients and 4 (6 %), 28 (44 %), 20 (31 %), 10 (16 %), and 2 (3 %), respectively, among female non-DM patients (Fig. 6). The frequency of advanced stage NASH was significantly higher in male DM patients than in male non-DM patients ($p < 0.05$).The rate of Stage 4 NASH was higher in female DM patients than in female non-DM patients; however, the difference was not significant ($p = 0.198$).

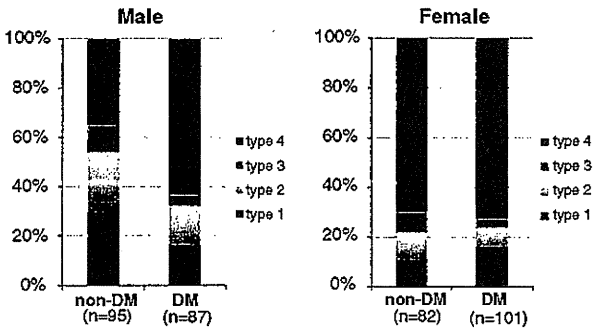


Fig. 5 Distribution of Matteoni's type classification in individual status of glucose metabolism among NAFLD patients. The frequency of type 4 NASH was significantly higher in male DM patients than in male non-DM patients ($p < 0.001$). The rate of type 4 NASH was high in both female DM and non-DM patients

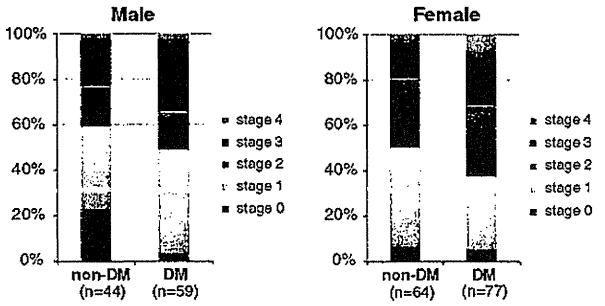


Fig. 6 Distribution of Brunt's stage in individual status of glucose metabolism among NASH patients. The frequency of advanced stage NASH was significantly higher in male DM patients than in male non-DM patients ($p < 0.05$). The rate of stage 4 NASH was higher in female DM patients than in female non-DM patients; however, the difference was not significant ($p = 0.198$)

HCC incidence in DM patients

In total, 67 (M 48, F 19) HCC cases (1.4 %) were reviewed (Table 2). HCC incidence was significantly higher in males

than in females. Five of 67 HCC patients consumed >60 g alcohol daily, and two of these five patients were anti-HCV Ab positive. HBsAg positivity, anti-HCV Ab positivity, and non-B non-C prevalence in the HCC patients was 8.6, 50.0, and 41.4 %, respectively. In a Japanese nationwide survey of 19,499 HCC patients [3], HBsAg positivity, anti-HCV Ab positivity, and non-B non-C prevalence was 15.0, 67.7, and 17.3 %, respectively. Non-B non-C prevalence was higher in our DM patients with HCC than in the nationwide HCC survey participants ($p < 0.001$). Mean PLT count in DM patients with HCC was as follows: HBsAg-positive patients, 12.4 ± 6.8; anti-HCV Ab-positive patients, 12.4 ± 5.6; and non-B non-C patients, 16.0 ± 7.0 ($\times 10^4/\mu L$); PLT count was significantly higher in the non-B non-C patients than in the anti-HCV-positive patients ($p < 0.05$). Mean BMI in these three patient groups was as follows: HBsAg-positive patients, 23.2 ± 5.1; anti-HCV Ab-positive patients, 22.8 ± 3.3; and non-B non-C patients, 27.2 ± 4.4 (kg/m^2); BMI was significantly higher in the non-B non-C patients than in the anti-HCV Ab-positive patients ($p < 0.001$).

Discussion

This is the first multicenter study, as per our knowledge, that clarifies the cause of liver injury in DM patients in Japan. Most Japanese HBV carriers are genotype C, acquired via perinatal vertical transmission or early childhood infection [12]. The HBV carrier rate in Japan is higher than that in western countries and significantly lower than that in other Asian countries [13]. In 1986, the Japanese government initiated a nationwide hepatitis B immunization program for infants born to HBV carrier mothers to prevent perinatal transmission. Consequently, the number of young serum HBsAg-positive individuals is extremely low. In our study, although the HBV carrier rate in DM patients was significantly higher than that in blood donors, 72 % of HBsAg-positive patients were serum HBV-DNA negative. Only 10 % of HBsAg-positive patients exhibited high serum HBV-DNA levels (≥4.0 log copies/ml), which is likely to induce hepatitis. These results indicate that a majority of DM patients who are HBV carriers may be asymptomatic.

Chronic hepatitis C may result in life-threatening complications, including cirrhosis and HCC. Worldwide, cirrhosis can be attributed to HBV (30 %) and HCV infection (27 %) [14]. The leading cause of cirrhosis among HBV and HCV sufferers and alcohol consumers varies with individual countries. A recent nationwide Japanese survey reported the etiology of cirrhosis in Japan as follows: HCV 60.9 %, HBV 13.9 %, alcoholism 13.6 %, primary biliary cirrhosis 2.4 %, NASH-related 2.1 %, and autoimmune hepatitis 1.9 % [15]. However, we must consider that hepatic triglycerides diminish with liver fibrosis progression in NASH patients (so-called "burned-out" NASH), resulting in difficulty in diagnosing NASH. Sixty-two percent of anti-HCV Ab-positive DM patients were HCV-RNA positive; these patients showed significantly higher serum ALT levels compared with HCV-RNA-negative patients. These results indicate that HCV infection is involved in the etiology of liver disease in DM patients.

There is no doubt that the positive rates of serum HBsAg and anti-HCV Ab in the general population are higher than in blood donors. Unfortunately, there were no data in the distribution of the rate of hepatitis virus carriers in each age group in Japan. In the present study, the positive rates of HBsAg and anti-HCV Ab in DM patients were significantly higher than that in blood donors. However, the present study demonstrated that most of HBsAg positive patients were negative for serum HBV DNA or had low serum HBV DNA levels and around one-third of anti-HCV Ab positive patients were negative for serum HCV RNA.

These results indicate the possibility that the frequency of hepatitis virus carriers in DM patients is higher than that in general population but no significant differences might be noted between DM patients and the general population.

Alcohol consumption is reportedly a significant factor associated with the risk of HCC development in patients with NASH-associated cirrhosis [16]. In our study, serum AST and ALT levels were comparable between drinkers consuming 20–59 g alcohol daily and nondrinkers. The ratio of heavy drinkers consuming >60 g alcohol daily was low (4.3 %) in our study. Moreover, drinking was not chosen as a variable related to elevated serum ALT levels. These results suggest that alcohol intake is not an important factor in the pathogenesis of liver disease in DM patients.

In our study, the frequency of anti-HCV Ab-positive DM patients was 5 %, whereas the serum HCV-RNA positivity rate in anti-HCV Ab-positive patients was 62 %. Therefore, the HCV carrier rate was calculated as 3 %. Since the proportion of HCV carriers and patients with elevated ALT levels were 3 % and up to 29 %, respectively, the influence of HCV infection is estimated to be no more than 10 % (3 % divided by 29 %) among DM patients with elevated ALT levels. There was no significant change in the number of DM patients with elevated ALT levels before and after elimination of HBV and/or HCV carriers and heavy drinkers. These results suggested that the major cause (up to 90 %) of liver injury in DM patients may be NAFLD.

In the present study, the frequency of advanced stage NASH was significantly higher in male DM patients than

in male non-DM patients. Neuschwander-Tetri et al. [17] reported that patients with advanced stage NASH were more likely to have DM. Mayaaki et al. [18] also examined the relationship between hepatic fibrosis stage and DM prevalence. In the mild fibrosis group, only 42 % were complicated with DM, whereas in the severe fibrosis group, the prevalence was as high as 71 % ($p = 0.020$). Lo et al. [19] reported that DM exacerbated diet-induced NASH fibrosis in mice. Therefore, DM may be an important factor in hepatic fibrosis development in NAFLD patients.

HCC frequency is significantly higher in obese and DM patients than in non-obese and non-DM patients [20, 21]. Recently, Tokushige et al. [22] reported on the backgrounds of Japanese HCC patients, and non-B non-C HCC accounted for 16 % of cases. A recent report has shown that NASH patients are likely to develop HCC in an earlier stage of fibrosis compared with chronic hepatitis C patients [23]. Our previous study analyzed 87 histologically proven NASH-HCC patients [24]; 37 % (20/54) of male HCC patients had a mild to moderate stage of liver fibrosis (F1 or F2); however, no female HCC patients were F1 stage, and only 15 % (5/33) were F2 stage. In the present study, DM patients with non-B non-C HCC exhibited a tendency to have higher PLT counts than those in DM patients with HCV-HCC, indicating that non-B non-C HCC is more likely to occur in DM patients with less advanced liver disease than in those with viral hepatitis.

In conclusion, HBsAg and anti-HCV Ab positivity rates were high; however, most of these patients were HBV-DNA negative or had low serum HBV-DNA levels. One-third of anti-HCV Ab-positive patients were HCV-RNA negative, and 4.3 % patients were drinkers whose ALT levels were comparable with those of nondrinkers. From these results, we conclude that up to 90 % of Japanese DM patients with liver injury may have NAFLD/NASH.

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Conflict of interest The authors declare that they have no conflicts of interest to disclose.

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＜短　報＞

岡山県における肝炎ウイルス検診陽性者の医療機関受診等に関する追跡調査

仁科　惣治[1)]　栗原　淳子[2)]　則安　俊昭[2)]　糸島　達也[3)]
山本　和秀[4)]　田中　純子[5)]　日野　啓輔[1)*]

緒言：平成14年から18年までの5年間に老人保健法に基づく保健事業として40歳から70歳までの受診対象者に対して5歳刻みで節目検診が行われ，また節目検診の対象者とならないが肝炎ウイルス感染の可能性が高い者（過去に肝機能異常を指摘されたことのある者，広範な外科的処置を受けたことのある者又は妊娠・分娩時に多量に出血したことのある者であって定期的に肝機能検査を受けていない者，基本健康診査の結果，ALT（GPT）値により要指導とされた者）に対して節目外検診が行われた．これらの検診により新たな肝炎ウイルス感染者が見いだされたが，その後の医療機関受診状況や肝炎に対する治療状況については，岡山県が平成17年度に市町村の協力の下で調査を行って以来，実態が把握されていないのが現状であった．田中らは「広島県における検診結果」として広島県12市町において聞き取り調査を行い，平成21年度報告書に報告している[5)]．それによると，HBVキャリアにおいては，回答率を考慮した医療機関受診率48%，把握されているHBVキャリア709名中440名から回答があった（回答率62.1%）．また，HCVキャリアにおいては，回答率を考慮した医療機関受診率65%，把握されているHCVキャリア630名中439名から回答があった（回答率69.7%）．また，医療機関受診率に関しては，HBVキャリアにおいては「現在受診中」が62%，「以前受診した」が15%，「受診していない」が23%であり，HCVキャリアにおいては「現在受診中」が80%，「以前受診した」が13%，「受診していない」が7%にすぎなかった．

そこで，厚生労働科学研究費補助金肝炎等克服緊急対策研究事業「肝炎ウイルス感染状況・長期経過と予後調査及び治療導入に関する研究」の一環として，岡山県における平成14年度から18年度までの節目・節目外検診で新たに見いだされた肝炎ウイルス感染者について，その後の医療機関受診状況や肝炎に対する治療状況を把握する目的でアンケートによる追跡調査を行ったので報告する．

対象と方法：対象は岡山県において平成14年度から18年度までの検診（節目・節目外）で肝炎ウイルス感染が判明した2,566人（B型974人，C型1,592人）のうち，調査可能であった24市町村において既に追跡調査等が行われていた肝炎ウイルス感染者を除いた1,352人（52.7%）（B型549人，C型803人）であった．調査用紙は各市町村より直接肝炎ウイルス陽性者へ郵送され，また一部の市町村（笠岡市58人，勝央町11人）においては保健師が直接聞き取り調査を行った．回答後の調査用紙は肝炎ウイルス陽性者から匿名の形で本研究の事務局である川崎医科大学肝胆膵内科学研究室へ直接郵送された．

アンケートは以下の項目について調査を行った．1. 在住市町村．2. 陽性と通知されたのはB型肝炎ウイルス（HBV）かC型肝炎ウイルス（HCV）か．3. 性別と年齢．4.「肝炎ウイルス感染の可能性が高い」と通知を受けて医療機関を受診したか否か．5. 受診していない場合その理由．6-1. 受診した場合は受診先がかかりつけ医か専門医療機関か．6-2. 受診先での診断名．7. 現在も通院を継続しているか否か．8. 通院を中止した場合はその理由．9. 治療を受けている場合は主な内容．

結果：調査を行った1,352人のうち716人（53%）より回答が得られた．このうち11人は既にウイルス性慢性肝炎として医療機関に通院していながら検診を受けており，8人は調査用紙の返送はあったものの無回答であり，1人は肝炎ウイルス陰性という回答であったため，

1）川崎医科大学肝胆膵内科学
2）岡山県保健福祉部健康推進課
3）岡山県医師会（岡山済生会総合病院）
4）岡山大学大学院医歯薬学総合研究科消化器・肝臓内科学
5）広島大学大学院医歯薬保健学研究院疫学・疾病制御学
*Corresponding author: khino@med.kawasaki-m.ac.jp
＄利益相反申告：山本　和秀，日野　啓輔⇄MSD（株）
＜受付日2012年9月18日＞＜採択日2012年11月16日＞

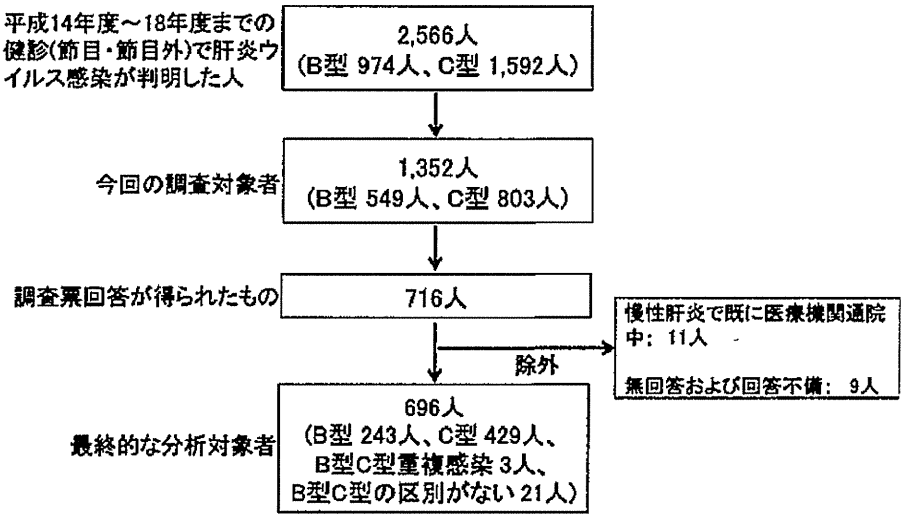

Fig. 1　今回の調査対象および分析対象者

この20人を除外した696人を分析対象とした．肝炎ウイルス別ではB型が243人，C型が429人，B型とC型の重複感染が3人，B型，C型の区別がないのが21人であった．平均年齢と性別（男/女）はB型が66.6歳（77/166），C型が72.1歳（142/287）であった．この検診は老人保健法に基づく健康診査の一環であることから，一般的な肝炎ウイルスキャリアにくらべて年齢層が高い集団であると考えられた．

医療機関受診率はアンケートに対する回答があった中で解析すると85%（211+397/716）であったが，調査表の回答者ではなく，調査表送付者に対する割合で算出したところ，B型が38.4%(211/549)，C型が49.4%(397/803)であった．したがって調査表の回答がなかった肝炎ウイルス陽性者はすべて受診していないと見なした数値である．「検診結果通知後受診しなかった」という回答者についてその理由を見てみると，「必要がないと思った」「肝機能に異常がない」「高齢である」「自覚症状がない」などの回答があった．受診したと答えたものの中の医療機関の受診先については，かかりつけ医の占める割合がB型で118人/211人（56%），C型で162人/397人（41%）であった．受診時の診断名はB型では211人中，肝機能異常なし，あるいは軽度異常程度が82.5%，慢性肝炎が11.4%，肝硬変・肝細胞癌が0.6%，残りの5.5%は不明であったのに対し，C型では397人中，肝機能異常なし，あるいは軽度異常程度が56.9%，慢性肝炎が26.2%，肝硬変・肝細胞癌が5.5%，残りの11.4%は不明であり，C型はB型にくらべて比較的進行した肝疾患を診断される割合が高かった．

医療機関受診者のその後の通院継続の有無については，B型が53.1%（129/211），C型が73.4%（314/397）の割合で通院を継続していた．通院を継続している場合の受療内容は，B型129人中の12.4%が核酸アナログ製剤の投与を受けており，C型314人中の23.3%がインターフェロン治療を受けていた．

通院を中止した理由では，「担当医から通院しなくてよいと言われた」というのが最も多く，B型の通院中断者82人のうち71.8%，C型の通院中断者83人のうち57%を占めた．

考察：今回岡山県で平成14年度から18年度にかけて行われた肝炎ウイルス検診の陽性者に対する追跡調査を行ったが，B型陽性者とC型陽性者では多少病態が異なるため，医療機関での診断名の割合が異なっていた．無症候性キャリアの割合が高いB型では肝機能異常なし，あるいは軽度異常程度が82.5%を占めており，またこの影響なのか医療機関通院継続率もC型に比べて低かった．田中らは「広島県における検診結果」として広島県12市町において聞き取り調査を行い，平成21年度報告書に報告している[1]．HBVキャリアにおいては，回答率を考慮した医療機関受診率48%，把握されているHBVキャリア709名中440名から回答があった(回答率62.1%)．また，HCVキャリアにおいては，回答率を考慮した医療機関受診率65%，把握されてい

るHCVキャリア630名中439名から回答があった（回答率69.7%）．それによると医療機関受診率に関しては，HBVキャリアにおいては「現在受診中」が62%，「以前受診した」が15%，「受診していない」が23%であった．一方HCVキャリアにおいては「現在受診中」が80%と高く，「以前受診した」が13%，「受診していない」は7%にすぎなかった．B型陽性者の通院中断の理由として，担当医から「通院しなくてもよい」と言われた割合が71.8%認めた点も今後の課題と考えられる．したがって，抗ウイルス療法が適切に行われていない可能性もあり，ウイルス肝炎治療のガイドラインに準じた治療の啓発をさらに推進する必要があると考えられた．現在，B型肝炎ウイルス感染者の肝発癌危険因子はウイルス量（HBV-DNA）であることが明らかにされているので[2]，トランスアミナーゼの値でフォローを中断するようなことは慎むべきであり，こうした点は肝臓専門医以外の医師にもっと広く啓発していく必要があると考えられる．

わが国の肝癌患者は高齢化が進んでおり，岡山県も例外ではない．今回の調査においてもアンケート回答者の平均年齢は高齢であった．肝炎ウイルス陽性の高齢者は肝癌の高危険群であり[3)~5)]，医療機関での経過観察あるいは治療が極めて重要である．これを実現するためには全県的な肝炎ウイルス陽性者の把握とともに地域に密接した保健活動を行政も含めてさらに推進していく必要がある．

結論：岡山県において平成14年度から18年度に実施された肝炎ウイルス検診（節目・節目外検診）で見出された陽性者について，その後の医療機関受診状況や受療状況を把握する目的で追跡調査を行った．肝炎ウイルス検診陽性者に対する医療機関への受診勧告や通院継続率の引き上げなどが今後の更なる課題と考えられた．

索引用語：ウイルス性肝炎，健康診断，追跡調査

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英文要旨

A follow-up survey of hepatitis virus carriers after notification of their infection in Okayama prefecture

Sohji Nishina[1)], Junko Kurihara[2)], Toshiaki Noriyasu[2)], Tatsuya Itoshima[3)], Kazuhide Yamamoto[4)], Junko Tanaka[5)], Keisuke Hino[1)]*

In Okayama prefecture we investigated by questionnaires whether hepatitis B virus (HBV) (n = 549) or hepatitis C virus (HCV) carriers (n = 803) consulted a doctor after they had been informed of their infection at their initial checkup for viral hepatitis between 2002 and 2006. The ratio of patients who consulted a doctor after notification of infection was 38.4% (211/549) of HBV carriers and 49.4% (397/803) of HCV carriers, respectively. Among those patients, 53.1% of HBV carriers and 73.4% of HCV carriers were on follow care at the start of this investigation. These results indicated a need to establish a more effective follow up system for hepatitis virus carriers following notification at the initial medical checkup.

Key words: virus hepatitis, medical checkup, follow-up survey

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1) Department of Hepatology and Pancreatology, Kawasaki Medical School
2) Public Health Policy Division, Department of Health and Social Welfare, Okayama Prefectural Government
3) Okayama Prefectural Medical Association (Okayama Saiseikai General Hospital)
4) Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences
5) Department of Epidemiology, Infectious Disease Control and Prevention, Hiroshima University, Institute of Biomedical and Health Sciences
*Corresponding author: khino@med.kawasaki-m.ac.jp