Table 1 Patients characteristics

Characteristic	Total	SVR	Non-SVR	*P**
n	650	210	440	
Sex (male/female)	405/245	134/76	271/169	0.604
Age (years)	57.4 ± 11.7	57.0 ± 11.9	57.6 ± 12.8	0.185
Height (cm)	162.8 ± 9.1	163.3 ± 9.2	162.1 ± 9.1	0.270
Body weight (kg)	63.1 ± 13.7	63.6 ± 13.9	62.1 ± 13.7	0.387
Body mass index	23.6 ± 3.1	23.7 ± 3.2	23.6 ± 3.2	0.654
Blood pressure (systolic, mmHg)	132 ± 17	130 ± 17	133 ± 18	0.334
Blood pressure (diastolic, mmHg)	78 ± 12	78 ± 11	79 ± 11	0.929
Hypertension (+/−)	152/498	48/162	104/336	0.844
HCV-genotype (1b/2a/2b/others)	389/159/56/46	92/84/19/15	297/75/37/31	<0.001
HCV RNA level (KIU/mL)	659 ± 508	435 ± 476	728 ± 532	<0.001
eGFR	85.2 ± 15.5	86.2 ± 15.9	84.7 ± 15.7	0.141
Fasting plasma glucose (mg/dL)	100 ± 31	99 ± 25	102 ± 34	0.888
Diabetes	149/501	42/168	107/333	0.232
Total cholesterol (g/dL)	156 ± 30	158 ± 38	154 ± 30	0.486
Triglyceride (mg/dL)	104 ± 46	108 ± 56	102 ± 45	0.764
Uric Acid (mg/dL)	5.6 ± 2.1	5.5 ± 2.1	5.7 ± 2.2	0.433
AST (IU/L)	62 ± 50	39 ± 19	73 ± 55	<0.001
ALT (IU/L)	68 ± 72	36 ± 20	80 ± 80	<0.001
Platelet ($\times 10^4/mm^3$)	11.6 ± 4.7	12.2 ± 5.0	11.3 ± 4.5	0.040
Frequencies of contrast imaging per year (≥1/<1)	252/398	28/182	224/216	<0.001
IFN monotherapy†/combination therapy‡	553/97	169/41	384/56	0.026

*Clinical differences between SVR group and Non-SVR group were evaluated by Wilcoxon rank sum test or Fisher's exact test.
†Outbreak of IFN monotherapy: recombinant IFNα 2a, 73 cases; recombinant IFNα 2b, 52 cases; natural IFNα, 278 cases; natural IFNβ, 150 cases; total dose of IFN = 572 ± 165 megaunit.
‡Outbreak of combination therapy: recombinant IFNα 2b+ribavirin, 29 cases, total dose of IFN = 502 ± 182 megaunit, total dose of ribavirin = 160 ± 68 g; peg IFNα 2b+ribavirin, 68 cases, total dose of peg IFN = 4.10 ± 1.08 mg, total dose of ribavirin = 202 ± 56 g.
Data are number of patients, median (range) or mean ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; IFN, interferon; SVR, sustained virological response.

2.67; 95% CI 1.34–5.32; $P. = 0.005$). The cumulative development rate for CKD based on difference of efficacy of the IFN therapy is shown in Figure 2. In addition to non-SVR, the four factors of aging, low eGFR, hypertension, and diabetes are high risk of developing the CKD. The development rates for CKD based on difference of age, eGFR, blood pressure, and blood glucose level at the starting time of follow-up are shown in Figure 3.

Incidence of end-stage CKD in cirrhotic patients with HCV

A total of six subjects (five male and one female) developed end-stage CKD during the follow-up period. The cumulative development rate of end-stage CKD was determined to be 0.4% at the 5th year, 1.6% at the 10th year and 2.8% at the 15th year by the use of the Kaplan–

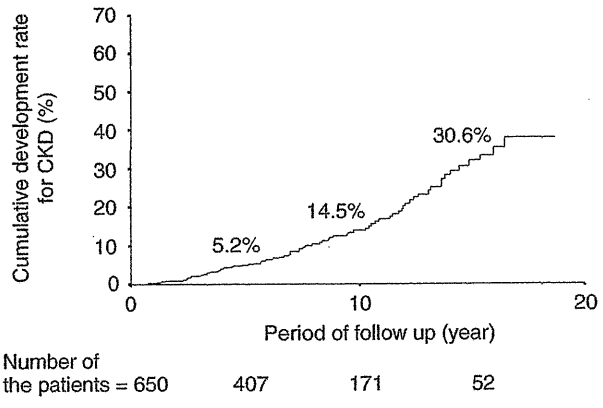

Figure 1 Cumulative development rate for chronic kidney disease (CKD) in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon.

Table 2 Predictive factors for chronic kidney disease (CKD) development

Variables	Univariate analysis		Cox-regression	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age, per 10 years	2.30 (1.72–3.12)	<0.001	2.32 (1.61–3.35)	<0.001
Sex (female/male)	0.90 (0.57–1.40)	0.628		
Body mass index (≥25/<25)	1.35 (0.72–2.50)	0.347		
HCV load(KIU/mL, ≥1000/<1000)	1.39 (0.80–2.38)	0.173		
Genotype (1/2)	1.19 (0.78–1.89)	0.436		
AST (IU/L, ≥50/<50)	1.63 (0.92–2.94)	0.097		
ALT (IU/L, ≥50/<50)	2.01 (1.13–3.57)	0.016		
Platelet ($\times 10^4/mm^3$, ≥15/<15)	0.70 (0.25–1.94)	0.487		
eGFR, per decrease of 10 mL/min/1.73 m^2	2.00 (1.56–2.56)	<0.001	1.66 (1.27–2.16)	<0.001
Uric acid (mg/dL, ≥7.0/<7.0)	1.43 (0.81–2.47)	0.225		
Triglyceride (mg/dL, ≥150/<150)	1.61 (0.62–3.70)	0.336		
Cholesterol (mg/dL, ≥220/<220)	1.22 (0.48–3.12)	0.678		
Diabetes (+/−)	2.76 (1.79–4.22)	0.001	1.79 (1.02–3.14)	0.042
Hypertension (+/−)	2.82 (1.80–4.39)	<0.001	2.00 (1.13–3.53)	0.017
Combination of ribavirin (+/−)	0.75 (0.36–1.58)	0.453		
Kind of IFN (beta/alpha)	0.91 (0.53–1.57)	0.729		
Efficacy (non-SVR/SVR)	2.10 (1.21–3.58)	0.008	2.67 (1.34–5.32)	0.005
Frequencies of contrast imaging per year (≥1/<1)	1.83 (1.17–2.87)	0.009		

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; HR, hazards ratio; IFN, interferon; SVR, sustained virological response.

Meier method (Fig. 4). The factors associated with the incidence of end-stage CKD in all 650 patients are shown in Table 3. There were no significant factors associated with the incidence of end-stage CKD as shown in Table 3.

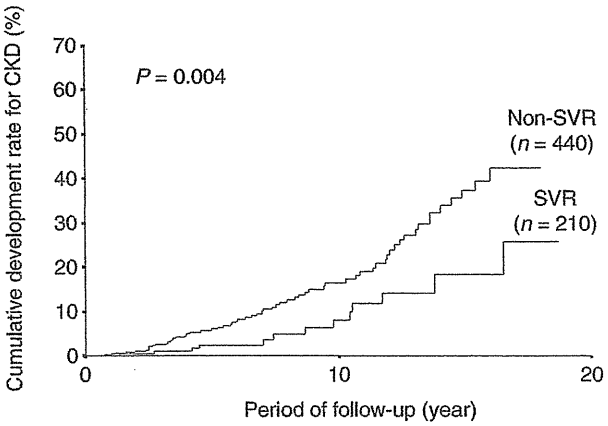

Figure 2 Cumulative development rate for chronic kidney disease (CKD) based on the difference of efficacy in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon.

DISCUSSION

WE HAVE DESCRIBED the development incidence for CKD and end-stage CKD after the termination of IFN therapy in HCV positive and liver cirrhotic patients treated with IFN. In the present study, the liver cirrhotic patients were enrolled to evaluate the new onset of CKD or end-stage CKD. Moreover, kidney damage has been reported in patients treated with IFN.[27] To exclude kidney damage originated from IFN-related side effects, patients with eGFR of ≥60 (mL/min per 1.73 m^2) for 3 months after the termination of IFN were enrolled in the present study. Our results indicate that the annual incidence for CKD as defined by a GFR of less than 60 mL/min per 1.73 m^2 for a prolonged follow-up after the termination of IFN therapy in HCV positive and cirrhotic patients is about 1.0–1.5% based on the development incidence for CKD at the 5th year and the 10th year. In addition, the annual incidence for end-stage CKD is one order of magnitude lower than that of a total of CKD.

Imai *et al.* have reported that about 20% of the Japanese adult population have stage 3 to 5 CKD by the use of database for 527 594 (male, 211 034; female, 316 560) participants obtained from the general adult population aged over 20 years who received annual

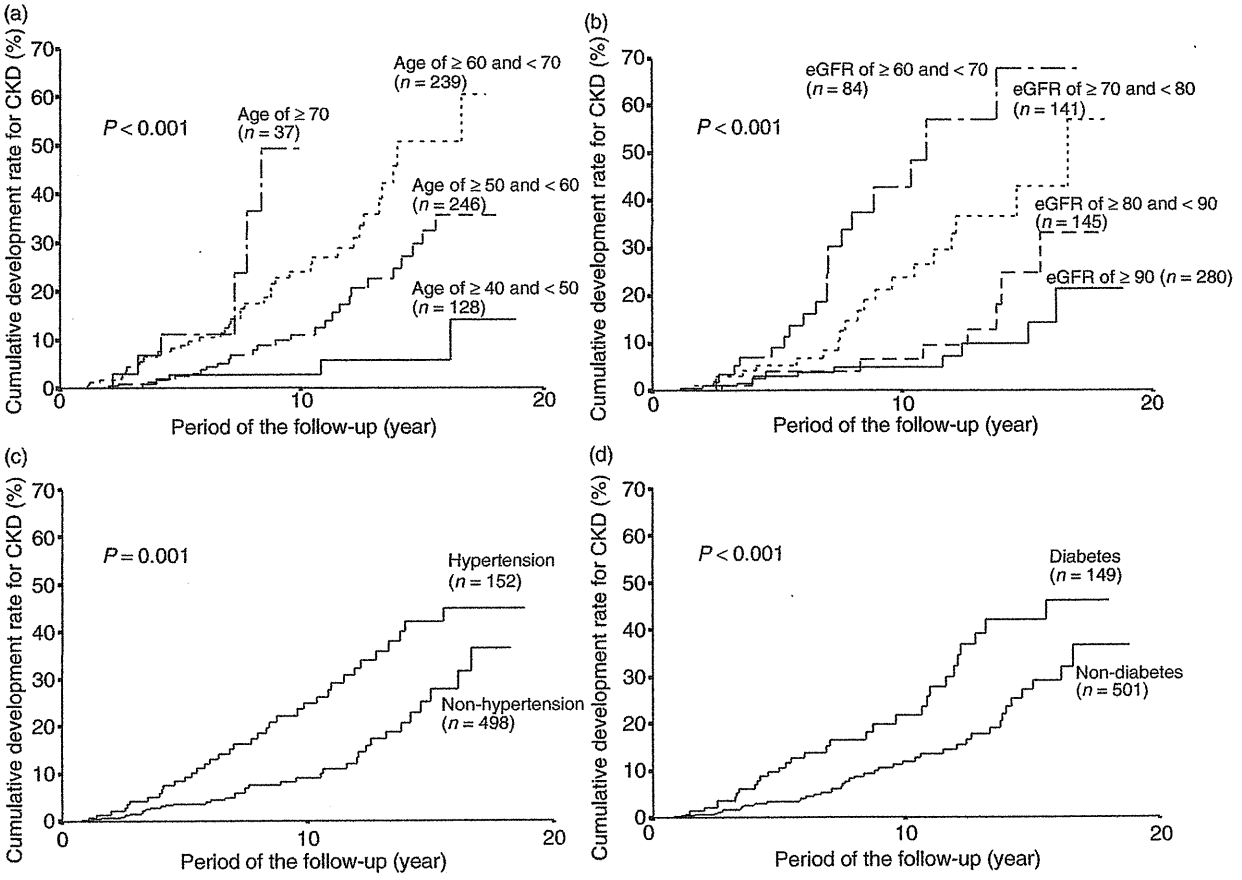

Figure 3 Cumulative development rate for chronic kidney disease (CKD) in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon: (a) Cumulative development rate for CKD based on difference of age; (b) Cumulative development rate for CKD based on the difference of estimated glomerular filtration rate (eGFR); (c) Cumulative development rate for CKD based on the difference of blood pressure; (d) Cumulative development rate for CKD based on the difference of glucose level.

health check programs in 2000–2004, from seven different prefectures in Japan. Next, the prevalence of CKD stage 3 in the study population, stratified by age groups of 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, and 80–89 years, were 1.4%, 3.6%, 10.8%, 15.9%, 31.8%, 44.0%, and 59.1%, respectively. Moreover, they provided that the prevalence of stage 4+5 was <0.2%. Our results agreed with Imai's report in the fact that end-stage CKD patients were few.

The present study was limited by a retrospective cohort trial. This cohort is over 10 years; hence, many patients had complications, such as diabetes and hypertension. However, the development of CKD was mainly evaluated based on the clinical characteristics at the initiation of follow-up. Second limitation of the study was that we defined CKD according to eGFR alone. Gener-

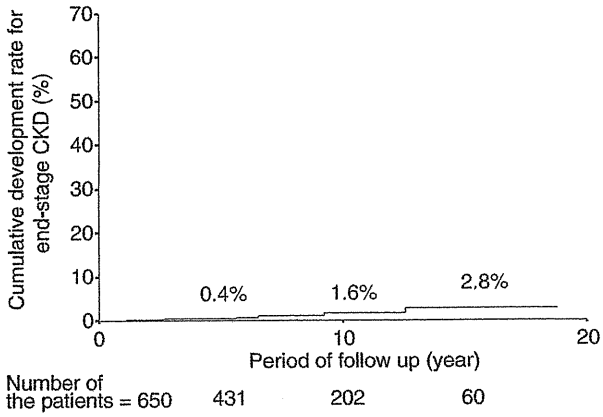

Figure 4 Cumulative development rate for end-stage chronic kidney disease (CKD) based on the difference of efficacy in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon.

Table 3 Predictive factors for end-stage chronic kidney disease (CKD) development

Variables	Univariate analysis	
	HR (95%CI)	*P*
Age, per 10 years	2.13 (0.86–5.30)	0.104
Sex (female/male)	0.24 (0.03–1.92)	0.182
Body mass index (≥25/<25)	0.80 (0.16–4.10)	0.782
HCV load (KIU/mL, ≥1000/<1000)	1.58 (0.37–6.67)	0.535
Genotype (1/2)	2.74 (0.66–11.50)	0.167
AST (IU/L, ≥50/<50)	1.45 (0.18–11.76)	0.730
ALT (IU/L, ≥50/<50)	1.89 (0.45–7.93)	0.382
Platelet ($\times 10^4$/mm^3, ≥15/<15)	0.67 (0.16–2.86)	0.586
eGFR, per decrease of 10 mL/min/1.73 m^2	1.70 (0.89–3.23)	0.105
Uric acid (mg/dL, ≥7.0/<7.0)	1.27 (0.23–6.96)	0.784
Triglyceride (mg/dL, ≥150/<150)	1.33 (0.15–11.87)	0.802
Cholesterol (mg/dL, ≥220/<220)	1.03 (0.12–8.67)	0.980
Diabetes (+/−)	1.89 (0.45–7.93)	0.382
Hypertension (+/−)	2.83 (0.70–11.41)	0.143
Combination of ribavirin (+/−)	0.88 (0.10–7.66)	0.908
Kind of IFN (beta/alpha)	2.08 (0.52–8.37)	0.300
Efficacy (non-SVR/SVR)	3.25 (0.40–26.4)	0.269
Frequencies of contrast imaging per year (≥1/<1)	3.72 (0.70–19.72)	0.123

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; HR, hazards ratio; IFN, interferon; SVR, sustained virological response.

ally, a recent definition of CKD also includes proteinuria.[28,29] Although the use of both eGFR and proteinuria might lead to a more accurate classification of CKD, we could not assess proteinuria in this study. Third, prescribed agents during the follow-up were not considered in the present study. However, therapy intervention is very important for protecting new development for CKD. In future, the intervention therapy for protecting the development of CKD should be evaluated. Finally, in the present study, patients were treated with different types of antivirus therapy (IFN monotherapy or combination therapy of IFN and ribavirin) for different durations (4 weeks to 52 weeks). This heterogeneity makes it slightly difficult to interpret the results of the study. On the other hand, the strengths of the present study are a long-term follow-up in the large numbers of patients included.

The present study shows several findings with regard to development incidence for CKD or end-stage CKD after the termination of IFN therapy for HCV positive and cirrhotic patients. First, SVR is effective for protecting the development incidence for CKD in HCV patients with liver cirrhosis. Though the role of HCV in the pathogenesis of aggravation of renal function remains speculative, the following possible mechanism have been reported: (i) systemic immune response to HCV infection mediated by cryoglobulins, HCV-antibody immune complexes, or amyloid deposition;[8,30,31] (ii) toll-like receptors increased expression in glomeruli induce immune response;[32] and (iii) insulin resistance and hyperinsulinemia cause excess intrarenal production of insulin-like growth factor-1 and transforming growth factor β, thus induce oxidative stress.[33] In addition, patients with liver cirrhosis might have the possibility of kidney damage such as hypovolemia due to fluid loss or hemorrhage, hepatorenal syndrome, and drug-induced renal failure. Second, in addition to non-SVR, the present study suggests that aging, low eGFR, hypertension, and diabetes enhanced the development of worsening renal function in cirrhotic patients with HCV infection after the termination of IFN. The repeated use of contrast imaging of computed tomography might worsen renal function. However, in the present study, SVR, aging, low eGFR, hypertension, and diabetes were the main predictive factors for the development of CKD compared to the repeated use of contrast imaging of computed tomography. The result that aging, hypertension and diabetes were associated with the development of worsening renal function agreed with several studies.[16–19]

In the present study, the predictive factors for end-stage CKD (stage 5) were not similar to those for CKD 3–5. The possible reason for this discrepancy is as follows. First, the number of patients who had progressed to end-stage CKD was six. Because of so few patients, we could not show the statistical significance in the predictive factors for end-stage CKD. Second, development of end-stage CKD might be robust to the several factors at the initiation of the follow-up. Development of end-stage CKD might be associated with the accidents during follow-up, such as the repeated use of contrast medium and hypovolemia due to bleeding. In fact, four of six patients who progressed to end-stage CKD had been given the repeated use of contrast medium. Next, whether HCV eradication in patients whose renal function progressed to stage of CKD 3–5 improves the mortality due to cardiovascular disease and stroke is a very important issue. However, this problem was not evaluated in the present study. This should be clarified by further examination.

In conclusion, our study suggests that the annual incidence for CKD among cirrhotic patients with HCV was determined to be about 1.0–1.5%. In addition, the annual incidence for end-stage CKD is one order of magnitude lower than that of CKD.

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Previous Chemoembolization Response after Transcatheter Arterial Chemoembolization (TACE) Can Predict the Anti-Tumor Effect of Subsequent TACE with Miriplatin in Patients with Recurrent Hepatocellular Carcinoma

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Key Words
Hepatocellular carcinoma · Miriplatin · Transcatheter arterial chemoembolization

Abstract
Aim: The purpose of this retrospective study was to evaluate the efficacy and safety of transcatheter arterial chemoembolization (TACE) with miriplatin in patients with unresectable hepatocellular carcinoma (HCC). ***Methods:*** From 2007 to 2010, 122 consecutive patients with unresectable HCC were treated by TACE with miriplatin-lipiodol suspension in our institute. Twenty-two patients (18%) had a solitary nodule and 100 patients (82%) had multiple nodules. Ninety-eight patients (80%) had a history of TACE. ***Results:*** Thirty-five of the 122 treated patients (29%) showed complete response (CR). And no serious complications were observed. Patients who had shown CR after previous TACE (pre-CR) were significantly more likely to show CR in the current study compared with patients who had shown less successful responses after previous TACE (56 vs. 20%, p = 0.003). Multivariate analysis revealed that response after previous TACE (pre-CR, risk ratio: 4.76; p = 0.035), tumor multiplicity (solitary, risk ratio: 9.69; p = 0.003), and injection artery (peripheral to segmental hepatic artery, risk ratio: 5.28; p = 0.040) were significant independent predictors associated with CR after TACE using miriplatin. ***Conclusion:*** In repetition of TACE treatment, switching the TACE agent from epirubicin or cisplatin to miriplatin offered a favorable treatment effect, especially in patients who had shown a CR after previous TACE.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases worldwide [1]. Since it is well known that more than 80% of HCC cases are associated with liver cirrhosis, a routine check-up including ultrasound for cirrhotic patients could potentially lead to the detection of early HCC [2–4]. Since curative therapies, including resection, liver transplantation, and percutaneous ablation (percutaneous ethanol injection and radiofrequency ablation) are applicable in only 30–40% of

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HCC patients, transcatheter arterial chemoembolization (TACE) has been recognized as an effective palliative treatment option for patients with advanced HCC [5–12].

Although many chemotherapeutic agents (e.g. doxorubicin, epirubicin, mitomycin C, and cisplatin) are used with the ethyl ester of iodized fatty acids from poppy-seed oil (Lipiodol Ultra-fluide; Laboratoire Guerbet, Aulnay-Sous-Bois, France) in TACE, the best choices for first- and second-line drugs remain uncertain [13–15]. Miriplatin (cis-[((1R,2R)-1,2-cyclohexanediamine-N,N′)bis(myristato)]-platinum(II)monohydrate; Dainippon Sumitomo Pharma Co., Osaka, Japan) is a novel lipophilic cisplatin derivative that can be suspended in lipiodol, a lipid lymphographic agent [16–19]. When lipiodol is injected into an artery feeding HCC nodules, it selectively accumulates in the tumor. Accordingly, a miriplatin-lipiodol emulsion is deposited within the HCC nodules and gradually releases active platinum compounds into tumor tissues. Clinical trials have demonstrated that miriplatin is effective in the treatment of HCC, but the efficacy of TACE using miriplatin for patients with recurrent HCC after TACE has not been evaluated [20, 21]. The purpose of this retrospective study was to evaluate the efficacy and safety of TACE using miriplatin for patients with HCC.

Patients and Methods

Study Population

From December 2007 to December 2010, 122 consecutive patients with unresectable HCC were treated by TACE with a miriplatin-lipiodol suspension at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. The study group consisted of 79 men and 43 women ranging in age from 48 to 87 years (median, 72 years). They included 11 patients (9%) positive for HBs-Ag, 103 patients (84%) positive for HCV antibody, and 8 patients (7%) negative for both. At the time of the miriplatin administration, median values were as follows: total bilirubin level = 1.1 mg/dl; serum albumin concentration = 3.3 g/dl; indocyanin-green retention rate at 15 min = 29%; prothrombin activity = 82.5%; alpha-fetoprotein (AFP) concentration = 31.2 ng/ml; and des-gamma-carboxyprothrombin (DCP) concentration = 53 AU/l. As for Child-Pugh classification, 92 patients (75%) were Class A and 30 patients (25%) were Class B. The clinical characteristics of the study group are summarized in table 1. The study protocol was approved by the ethics committee of our hospital, and written informed consent was obtained from all participating patients.

Hepatocellular Carcinoma

Before treatment with miriplatin, all patients underwent a comprehensive evaluation consisting of medical history, physical examination, measurement of tumor size, performance status, chest radiograph, liver-imaging studies (dynamic computerized tomography [dynamic CT], ultrasonography [US], digital-subtraction angiography [DSA]), complete blood count, and blood chemistry. Diagnosis of HCC was established based on the findings of dynamic CT, US and DSA. Patients who had extrahepatic metastasis of HCC or other malignancies were excluded.

Tumor profiles and TACE treatment history for the study group are summarized in table 2. Twenty-two patients (18%) had a solitary nodule and 100 patients (82%) had multiple nodules. The median diameter of the largest tumor was 20 mm (range 10–100 mm). Ninety-eight patients (80%) had a history of TACE. Thirty-seven patients had received cisplatin, and 80 patients had received epirubicin. Among these patients, the median number of

Table 1. Demographic characteristics and pretreatment assessments of 122 patients who underwent TACE using a miriplatin/lipiodol suspension for unresectable HCC

Number of cases	122
Age, years	72 (48–87)
Gender, male	65%
Etiology, HCV/HBV/others	103/11/8
Child-Pugh Class, A/B/C	92/30/0
ICG-R15, %	29 (4–78)
Albumin, g/dl	3.3 (2.0–4.2)
Total bilirubin, mg/dl	1.1 (0.4–4.9)
Prothrombin activity, %	82.5 (45.7–123.1)
Platelet, $\times 10^3/\mu l$	93 (29–282)
AFP, ng/ml	31.2 (1.8–152,800)
DCP, AU/l	53 (6–65,290)

HCV = Hepatitis C virus; HBV = hepatitis B virus; ICG-R15 = indocyanine-green retention rate at 15 min.

Variables are expressed as medians with ranges in parentheses.

Table 2. Tumor profiles, treatment history, and study drug dosages of 122 patients who underwent TACE using miriplatin for unresectable HCC

Tumor size, mm	20 (10–100)
Intrahepatic multiplicity, solitary	22 (18%)
Number of tumors	4 (1–100)
Presence of portal vein invasion	3 (2%)
History of TACE	98 (80%)
History of TACE with epirubicin	80 (66%)
History of TACE with cisplatin	37 (30%)
Median interval between previous TACE and miriplatin administration, months	4 (1–41)
Dosage of miriplatin, mg	80 (20–120)
Dosage of lipiodol, ml	3 (1–6)
Injection from peripheral to segmental branch of the hepatic artery	22 (18%)

Variables are expressed as medians with ranges in parentheses or number of cases.

TACE procedures was four (range 1–13), and the median interval between previous TACE and miriplatin administration was 4 months (range 1–41 months).

Treatment Protocol

Patients were hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and the catheter was inserted superselectively into the hepatic artery that supplied the target tumor for injection of the miriplatin-lipiodol suspension and 1-mm gelatin cubes (Gelpart; Nippon Kayaku, Tokyo). The miriplatin-lipiodol suspension was administrated slowly under careful fluoroscopic guidance. The dose of miriplatin/lipiodol was determined according to tumor size and the degree of liver dysfunction.

Assessment of Therapeutic Effects

The effect of chemotherapy was evaluated by dynamic CT 1 to 3 months after TACE with miriplatin, and was based on the change in the maximum diameter of the viable target lesions (i.e. showing enhancement in the arterial phase). Response categories, according to the criteria of Modified Response Evaluation Criteria in Solid Tumors (mRECIST) [22], are as follows: complete response (CR) = disappearance of any intratumoral arterial enhancement in all target lesions; partial response (PR) = at least a 30% decrease in the sum of diameters of viable target lesions; stable disease (SD) = any cases that do not qualify for either PR or progressive disease; and progressive disease (PD) = an increase of at least 20% in the sum of the diameters of viable target lesions.

Toxicity Evaluation

Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria (version 4.0). Within 2 weeks before TACE with miriplatin, and at 3 to 7 days (three times during this period) and at 1 month afterward, the following toxicity evaluations were made: hematological assessments (i.e. leukocyte and thrombocyte counts) and clinical chemistry assessments (i.e. serum aspartate aminotransferase [AST], serum alanine aminotransferase [ALT], albumin, total bilirubin, serum creatine, and prothrombin activity).

Statistical Analysis

The distribution of subject characteristics was assessed by the chi-square test or the Mann-Whitney's U test, as appropriate. Multivariate logistic regression analysis was used to evaluate significant factors for CR by TACE with miriplatin. All variables are expressed as mean (range). All tests were 2-sided, and p values less than 0.05 were considered statistically significant. Statistical analyses were performed using SPSS, version 13.0 (SPSS Inc., IBM, Somers, N.Y., USA).

Results

Dosing of Study Drugs

Table 2 summarizes the profiles and study drug data of 122 HCC patients who were treated with miriplatin. The median dosage of miriplatin was 80 mg (range 20–120 mg), and the median dosage of lipiodol was 3 ml (range 1–6 ml). Twenty-two patients (18%) were injected with the miriplatin-lipiodol suspension from the peripheral to the segmental branch of the hepatic artery. Thirty patients (25%) were injected with the miriplatin-lipiodol suspension from the anterior or posterior segmental branch of the right hepatic artery. Sixty-six patients (54%) were injected with the miriplatin-lipiodol suspension from the right or left branch of the hepatic artery. And 4 patients (3%) were injected with the miriplatin-lipiodol suspension from the proper hepatic artery.

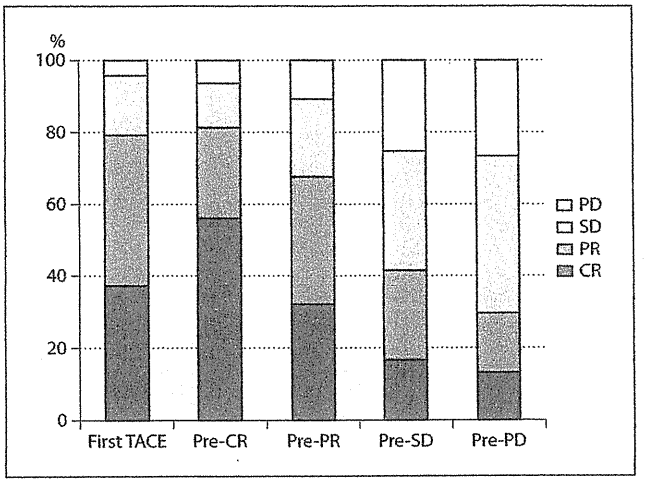

Fig. 1. The efficacy of TACE using miriplatin in patients with HCC according to response to previous TACE. Abbreviations used in the figure: CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease. First TACE group (n = 24): patients who received TACE for the first time. pre-CR group (n = 16): patients who showed CR after previous TACE. pre-PR group (n = 28): patients who showed PR after previous TACE. pre-SD group (n = 24): patients who showed SD after previous TACE. pre-PD group (n = 30): patients who showed PD after previous TACE.

Treatment Effects

Thirty-five of the 122 treated patients (29%) showed CR, 35 patients (29%) showed PR, 33 patients (27%) showed SD, and 19 patients (15%) showed PD. Overall, 58% of patients showed an objective response (i.e. CR or PR).

Treatment Effects according to Previous TACE Effect

The efficacy of TACE using miriplatin according to the treatment effect of previous TACE was as follows (and is illustrated in fig. 1). For the first TACE group (patients who received TACE for the first time), 9 of 24 patients (38%) showed CR; for the pre-CR group (patients who

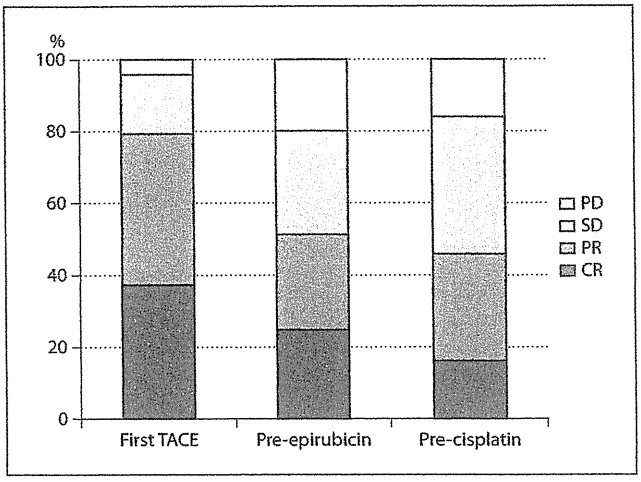

Fig. 2. The efficacy of TACE using miriplatin in patients with HCC according to previous TACE agent. Abbreviations used in the figure: CD = complete response; PR = partial response; SD = stable disease; PD = progressive disease. First TACE group (n = 24): patients who received TACE for the first time. Pre-cisplatin group (n = 37): patients who had received TACE using cisplatin. Pre-epirubicin group (n = 80): patients who had received TACE using epirubicin.

showed CR response after previous TACE), 9 of 16 patients (56%) showed CR; for the pre-PR group (patients who showed PR response after previous TACE), 9 of 28 patients (32%) showed CR; for the pre-SD group (patients who showed SD response after previous TACE), 4 of 24 patients (17%) showed CR; and for the pre-PD group (patients who showed PD response after previous TACE), 4 of 30 patients (13%) showed CR.

Treatment Effects according to Previous TACE Agent

In patients who had received TACE using epirubicin, 20 of 80 patients (25%) showed CR and 21 of 80 patients (26%) showed PR. In patients who had received TACE using cisplatin, 6 of 37 patients (16%) showed CR and 11 of 37 patients (30%) showed PR. In each of the above groups, the objective response rate (sum of CR and PR) was significantly lower than that in patients who received their first TACE ($p = 0.015$ and $p = 0.010$, respectively), as illustrated in figure 2.

Univariate analysis identified the following six factors as influencing the rate of CR: response after previous TACE (pre-CR group vs. other groups, $p = 0.005$), tumor multiplicity (solitary vs. multiple, $p < 0.0001$), gamma-GTP concentration (≤40 vs. >40 IU/l, $p = 0.037$), AFP concentration (≤40 vs. >40 ng/ml, $p = 0.042$), DCP concentration (≤50 vs. >50 AU/l, $p = 0.003$), and injection artery (peripheral to segmental hepatic artery vs. right or left hepatic artery and proper hepatic artery, $p = 0.001$). These parameters were entered into multivariate logistic regression analysis, which revealed that response after previous TACE (pre-CR group vs. other groups, risk ratio: 4.76; 95% CI: 1.11–20.37; $p = 0.035$), tumor multiplicity (solitary vs. multiple, risk ratio: 9.69; 95% CI: 2.18–42.92; $p = 0.003$), and injection artery (peripheral to segmental hepatic artery vs. right or left hepatic artery and proper hepatic artery, risk ratio: 5.28; 95% CI: 1.07–25.95; $p = 0.040$) were significant independent predictors associated with CR after TACE using miriplatin (table 3).

Adverse Effects

Fever, anorexia, and elevation of serum transaminase levels were observed in most patients after miriplatin administration (table 4). The following Grade 4 events were observed: decreased neutrophil count in 1 patient (1%), increased AST in 4 patients (3%), and increased ALT in 1 patient (1%); all these cases resolved within 2 weeks. In this study group, no vascular complications of the hepatic artery were observed. No other serious complications or treatment-related deaths were observed after miriplatin administration.

Discussion

TACE is most widely performed in patients with HCC who are not eligible for curative therapy. The survival benefit of TACE has been confirmed by randomized controlled trials and meta-analyses. Various anti-cancer drugs, such as doxorubicin, epirubicin, mytomycin C, cisplatin, and neocarzinostatin, have been used as TACE agents for the treatment of HCC. However, the most effective and least toxic TACE protocol for HCC has yet to be identified [13–15].

Although TACE can be repeated in most patients, good therapeutic efficacy cannot be expected when the same anti-cancer drug is used more than once since various types of resistance to therapy can develop during repetition of TACE. Platinum derivatives are frequently administered to patients with advanced HCC that is unresponsive to anthracycline and antibiotic drugs [23, 24]. Miriplatin was developed as a lipophilic platinum complex in an effort to produce a superior anti-tumor effect in HCC with lower toxicity compared with cisplatin [16–

Table 3. Univariate and multivariate analysis of predictors of complete necrosis (logistic regression analysis)

Category	Univariate		Multivariate	
	Hazard ratio (95% CI)	p value	Hazard ratio (95% CI)	p value
Tumor multiplicity, solitary vs. multiple	8.57 (3.08–23.8)	<0.0001	9.69 (2.19–42.9)	0.003
Response by pre-TACE, pre-CR vs. others	4.91 (1.59–15.1)	0.005	4.76 (1.11–20.3)	0.035
Injection artery, peripheral to segmental hepatic artery vs. others	2.50 (0.96–6.48)	0.001	5.28 (1.07–25.9)	0.040
DCP, ≤50 vs. >50 AU/l	4.04 (1.61–10.13)	0.003	3.55 (0.99–12.6)	0.051
gamma-GTP, ≤40 vs. >40 IU/l	2.39 (1.05–5.44)	0.037		
AFP, ≤40 vs. >40 ng/ml	2.50 (1.03–6.06)	0.042		

Table 4. Adverse effects after miriplatin administration

Grade:	1	2	3	4
White blood cell decreased	1 (1%)	27 (22%)	7 (6%)	0
Neutrophil count decreased	2 (2%)	21 (17%)	5 (4%)	1 (1%)
Anemia	40 (33%)	21 (17%)	3 (2%)	0
Platelet count decreased	72 (59%)	21 (17%)	11 (9%)	0
AST increased	55 (45%)	23 (19%)	30 (25%)	4 (3%)
ALT increased	54 (44%)	12 (10%)	19 (16%)	1 (1%)
Fever	67 (55%)	14 (11%)	0	0
Anorexia	56 (46%)	1 (1%)	0	0
Nausea	23 (19%)	0	0	0
Abdominal pain	22 (18%)	4 (3%)	0	0
Hepatic infection	0	0	1 (1%)	0

Values denote numbers of subjects. Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria version 4.0.

19]. Miriplatin-lipiodol suspension is a stable colloidal emulsion that is deposited within HCC tumors, where it gradually releases active derivatives of miriplatin.

According to pharmacokinetic studies, the plasma concentration of total platinum is much lower in patients treated with miriplatin compared with that in patients treated with intra-arterial cisplatin: the Cmax is approximately 300-fold lower and the Tmax roughly 500-fold longer for miriplatin than the corresponding values for intra-arterial cisplatin.

Miriplatin/lipiodol releases 1,2-diaminocyclohexane platinum (II) dichloride (DPC) as its active platinum compound, which binds to nuclear DNA and mediates miriplatin/lipiodol cytotoxicity. In a cisplatin-resistant rat hepatoma cell-line model, cross-resistance to DPC was not observed [25].

Prior to the current study, clinical trials have shown that miriplatin is effective for the treatment of HCC, but the efficacy of switching the TACE anti-cancer drug from epirubicin or cisplatin to miriplatin for a repeat TACE had not been evaluated.

In the present study, having a low number of tumors (solitary vs. multiple), receiving the treatment injection in the peripheral to segmental hepatic artery, and having shown complete tumor necrosis after prior TACE (pre-CR group) were highly correlated with complete tumor necrosis after TACE with miriplatin. A previous CR may be a surrogate marker for other factors, such as tumor sensitivity to anti-cancer agents and intra-hepatic metastasis. Among the 54 patients in this study who had shown no change or disease progression after previous TACE (pre-SD and pre-PD groups), 19 patients (35%) showed an

objective response by switching the TACE agent from epirubicin or cisplatin to miriplatin.

In repetition of TACE, vascular complications can cause development of parasitic feeding arteries for liver cancers leading to insufficient tumor embolization; rapid tumor growth may follow. In the present study, no vascular complications or other serious adverse events were observed. These results suggest that miriplatin may be used effectively and safely as a second-line TACE drug for recurrent HCC after TACE.

Previous studies reported that complete tumor necrosis after TACE offered favorable long-term survival outcomes to HCC patients [7, 26]. In the current study, miriplatin administration was associated with a beneficial tumor response even in recurrent HCC after TACE. These results suggest that miriplatin administration may offer a favorable prognosis for recurrent HCC after TACE.

Conclusion

In repetition of TACE in HCC patients, switching the TACE agent from epirubicin or cisplatin to miriplatin offered a favorable treatment effect, especially in patients who had shown CR after previous TACE. These results suggest that miriplatin may be used effectively and safely as a second-line TACE drug for recurrent HCC after TACE.

Disclosure Statement

The following authors have received honoraria (lecture fee) from Dainippon Sumitomo Pharma Co., Ltd, Osaka, Japan: Hiromitsu Kumada, MD; Kenji Ikeda, MD; Yasuji Arase, MD; Yoshiyuki Suzuki, MD; Fumitaka Suzuki, MD; and Norio Akuta, MD.

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INTERNAL MEDICINE

ORIGINAL ARTICLE

The Development of Chronic Kidney Disease in Japanese Patients with Non-alcoholic Fatty Liver Disease

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Abstract

Objective Chronic kidney disease (CKD) is present in patients with nonalcoholic fatty liver disease (NAFLD). The aim of this retrospective study was to assess the cumulative development incidence and predictive factors for new onset of CKD in Japanese patients with NAFLD.

Methods A total of 5,561 NAFLD patients without CKD were enrolled. CKD was defined as either an estimated glomerular filtration rate of <60 mL/min/1.73 m^2 or dipstick proteinuria ($\geq +1$). A blood sample and a urine sample were taken for routine analyses during follow-up. The mean observation period was 5.5 years. The primary goal is the new development of CKD. Independent factors associated with new development of CKD were analyzed by using the Kaplan-Meyer method and the Cox proportional hazards model.

Results Of 5.561 NAFLD patients, 263 patients developed CKD. The cumulative development rate of CKD was 3.1% at the 5th year and 12.2% at the 10th year. Multivariate Cox proportional hazards analysis showed that CKD development in patients with NAFLD occurred when patient had low level of GFR of 60-75 mL/min/1.73 m^2 [hazard ratio:2.75; 95% confidence interval (CI) =1.93-3.94; p<0.001], age of ≥50 years (hazard ratio: 2.67; 95% CI=2.06-3.46; p<0.001), diabetes (hazard ratio: 1.92; 95% CI=1.45-2.54; p<0.001), hypertension (hazard ratio: 1.69; 95% CI=1.25-2.29; p<0.001), and elevated serum gamma-glutamyltransferase of ≥109 IU/L(hazard ratio: 1.35; 95% CI=1.02-1.78; p=0.038).

Conclusion Our retrospective study indicates that the annual incidence of CKD in Japanese patients with NAFLD is about 1.2%. Five factors of low eGFR level, aging, type 2 diabetes, hypertension, and elevated gamma-glutamyltransferase, increases the risk of the development of CKD.

Key words: nonalcoholic fatty liver disease, chronic kidney disease, gamma-glutamyltransferase

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the more common causes of chronic liver disease in Western world (1-4) and in many Asian nations (5, 6). NAFLD is considered to be the liver component of metabolic syndrome (7-9). It is associated with obesity, dyslipidemia, pituitary dysfunction, hypertension, sleep apnea, and type 2 diabetes mellitus (T2DM) (10-16). Moreover, NAFLD often causes cardiovascular disease and stroke (17, 18). Thus, NAFLD is emerging as a new significant health problem in many countries.

On the other hand, there has been a recent dramatic in-

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crease in the prevalence of end-stage renal disease (ESRD) in USA and Asia (19-22). Chronic kidney disease (CKD) often progresses to ESRD with its attendant complications. CKD, a disease entity including mild to ESRD due to any etiology, was defined as an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 and/or the presence of proteinuria (21). Recently, metabolic syndrome and NAFLD have been reported to enhance the new onset of CKD (23, 24). Although there is growing evidence to support the concept that metabolic syndrome is a risk factor for developing CKD, little research has been done to evaluate whether NAFLD is associated with the long-term development of CKD.

The present cohort study was initiated to investigate the cumulative incidence and risk factors of CKD after long-term follow-up in patients with NAFLD. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

Methods

Patients

The number of Japanese patients who were diagnosed with fatty liver by ultrasonography (25) between January 1997 and December 2007 in the Department of Hepatology and/or Health Management Center, Toranomon Hospital, Tokyo, Japan was 9,120. Of these, 5,561 Japanese patients satisfied with the following enrolled criteria; 1) no evidence of CKD based on eGFR calculated with serum creatinine level (eGFR≥60 [mL/min/1.73 m^2]); 2) the absence of proteinuria (≥ +1); 3) current and past daily alcohol intake of <20 g/day; 4) negativity for hepatitis B surface antigens (HBsAg), hepatitis C virus antibodies, antinuclear antibodies, or antimitochondrial antibodies in serum, as determined by radioimmunoassay, enzyme-linked immunosorbent assay or spot hybridization; 5) no underlying neoplasm or systemic disease, such as systemic lupus erythmatosus, rheumatic arthritis; 6) no evidence of nodules of hepatocellular carcinoma as shown by ultrasonography and/or computed tomography. Patients with the above criteria were enrolled regardless of whether the serum level of aminotransferase was normal or abnormal. Patients with any of the following criteria were excluded from the study: 1) illness that could seriously reduce their life expectancy, 2) findings suggestive of other chronic liver disease, and 3) refusal to be followed up after the diagnosis of NAFLD. A total of 3,559 out of 9,120 patients were excluded based on the following findings; 169 had a dipstick-positive proteinuria; 1,685 had an eGFR of <60 mL/min/1.73 m^2; 2,098 had alcohol intake of ≥20 g/day; 133 had positive serologic findings for either hepatitis B or C virus, a reported history of known liver disease, or decompensated liver cirrhosis; 36 had a history of malignancy; 26 had a history of cardiovascular disease; 11 refused the participation of prospective follow-up. Because some individuals were excluded for multiple reasons, the total number of eligible patients for the study was 5,561.

Patients were classified into three groups according to fasting plasma glucose (FPG): 1) those with FPG level of < 110 mg/dL (normal glucose group), 2) those with FPG level of 110-125 mg/dL (pre-diabetes group), and 3) those with FPG level of ≥126 mg/dL (diabetes group) (25). Patients were regarded as hypertension by the confirmation of blood pressure ≥140 mmHg systolic and/or ≥90 mmHg diastolic.

The primary goal was the new onset of CKD in patients with NAFLD. The end-point was defined as the first eGFR <60 mL/min/1.73 m^2 or dipstick proteinuria (≥ +1) for more than three months. Serum creatinine level was also measured using an enzymatic method, and the GFR was estimated from the Japanese Society of Nephrology CKD Practice Guide; eGFR (mL/min/1.73 m^2) =194× (serum creatinine level [mg/dL]) -1.094× (age [y]) -0.287. The product of this equation was multiplied by a correction factor of 0.739 for women. CKD and its stages were defined from estimated eGFR of <60 mL/min/1.73 m^2 or dipstick proteinuria (≥ +1) as follows: stage I, eGFR≥90 and proteinuria (≥ +1); stage II, 90>eGFR≥60 and proteinuria (≥ +1); stage III, 60>eGFR≥30; stage IV, 30>eGFR≥15; and stage V, 15>eGFR. Patients with stage III-V were regarded as having CKD regardless of the absence of other markers of kidney damage (21, 22).

All of the studies were performed retrospectively by collecting and analyzing data from the patient records. This study was approved by Institutional Review Board of our hospital.

Medical evaluation

Fatty liver was diagnosed by the presence of an ultrasonographic pattern consistent with bright liver with stronger echoes in the hepatic parenchyma than in the renal or spleen parenchyma (26). Ultrasonography test was performed with a high-resolution, real-time scanner (model SSD-2000; Aloka Co., Ltd, Tokyo Japan. Mode Logic-700 MR; GE-Yokokawa Medical Systems, Tokyo, Japan). Body weight was measured in light clothing and without shoes to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm. Height and weight were recorded at baseline and the body mass index (BMI) was calculated as weight (in kg) / height (in m^2). All of the patients were interviewed in the Toranomon Hospital using a questionnaire that gathered information on demographic characteristics, medical history, and heath-related habits including questions on alcohol intake at the time of diagnosis of fatty liver.

Laboratory investigation

At the first consultation anti-HCV and HBsAg were examined. Anti-HCV was detected using a third-generation enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, IL). HBsAg was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI). Anti-HBs was not evaluated in the present study. Serum creatinine concentration was measured by a modified Jaffe method (creatinine

Table 1. Characteristics of Subjects Enrolled

	Total
Number of cases	5561
Age (years)	48.0±8.4
Sex(male/female)	4916/645
Systolic blood pressure(mmHg)	134±18
Diastolic blood pressure(mmHg)	76±10
Hypertension(+)	725(13.0%)
Height(cm)	167.8±7.3
Body Weight (kg)	70.7±9.9
BMI (kg/m^2)	25.1±2.8
Smoking (+)	1028 (18.5%)
FPG(mg/dL)	104.7±24.8
Glucose status (Normal/ preDM/DM)	4436(79.8%)/667(12.0%)/458(8.2%)
eGFR (mL/min/1.73m^2)	74.6±11.9
WBC(×10^3/mm^3)	5.8±1.5
Hemoglobin (g/dL)	15.1±1.2
Platelet (×10^4/mm^3)	23.1±5.0
Triglyceride (mg/dL)	164±117
Total cholesterol (mg/dL)	210±34
HDL cholesterol (mg/dL)	48.1±11.9
Total Protein(g/dL)	7.5±0.4
Albumin (g/dL)	4.2±0.3
Uric Acid (mg/dL)	6.2±1.3
AST (IU/L)	29.2±16.4
ALT (IU/L)	37.5±27.0
GGT(IU/L)	78.2±81.0
Follow-up period (years)	5.5±4.8

Data are number of patients (percent) or mean ± standard deviation
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DM, diabetes mellitus, eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GGT, gamma-glutamyltransferase; HDL, high density lipoprotein; WBC, white blood cell;

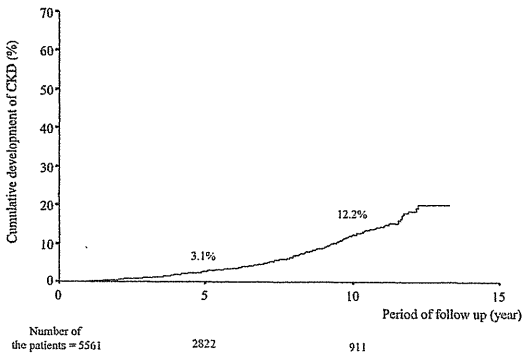

Figure 1. Cumulative development rate of CKD in 5,561 patients with NAFLD.

HR, Wako Pure Chemicals Industries, Ltd, Osaka, Japan) using an autoanalyzer (Hitachi 7350, Hitachi Ltd, Tokyo, Japan or RX-20, JEOL Ltd., Tokyo, Japan). Proteinuria was tested using dipsticks (Ames Hemacombistics; Bayer-Sankyo Ltd, Tokyo, Japan). A test result of $\geq +1$ was defined as positive.

Follow-up

Starting time of follow-up was the day that the fatty liver was confirmed by ultrasonography. After that, patients were followed up monthly to six-monthly in the Toranomon hospital. A blood sample and a urine sample were taken for routine analyses. Four hundred and ninety-two patients were lost to follow-up. Because the appearance of CKD was not identified in these 492 patients, they were considered as censored data in statistical analysis (27).

Statistical Analysis

The cumulative appearance rate of CKD was calculated from the starting time of follow-up to the development of CKD by using the Kaplan-Meier method. Differences in the development of CKD were tested using the log rank test. The Cox proportional hazard model analyzed independent factors associated with the development rate of CKD. The following variables were analyzed for potential covariates for incidence of CKD: age, BMI, T2DM, hypertension, and levels of eGFR, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), total protein, triglyceride (TG), total cholesterol level, high density lipoprotein (HDL) cholesterol uric acid, hemoglobin, white blood cell, platelet at the time of diagnosis of NAFLD. A p value of less than 0.05 was considered significant. Data analysis was performed using the computer program SPSS package (SPSS 11.5 for Windows, SPSS, Chicago, IL).

Results

Patients' characteristics

Table 1 shows the characteristics in the 5,561 patients diagnosed as NAFLD in the present study. The mean age was 48 years. The mean BMI was 25.1. Patients with hypertension accounted for 13.0% and patients with T2DM accounted for 8.2%. The eGFR level was 74.6±11.9 mL/min/1.73 m^2. The mean follow-up period was 5.5 years.

Incidence of CKD in Patients with NAFLD

Of 5.561 NAFLD patients, 263 developed CKD. Figure 1 shows that the cumulative development rate of CKD was 3.1% at the 5th year and 12.2% at the 10th year in all patients with NAFLD. Cox proportional hazards analysis showed that CKD development in NAFLD patients occurred when patient had eGFR of 60-75 mL/min/1.73 m^2 [hazard ratio:2.75; 95% confidence interval (CI) =1.93-3.94; p< 0.001], age of $\geq$50 years (hazard ratio:2.67; 95% CI =2.06-3.46; p<0.001), T2DM (hazard ratio:1.92; 95% CI=1.45-2.54; p<0.001), hypertension (hazard ratio:1.69; 95% CI= 1.25-2.29; p<0.001), and elevated serum GGT (hazard ratio: 1.35; 95% CI=1.02-1.78; p=0.038) at the initiation of follow up (Table 2).

Figure 2 shows the cumulative development rate of CKD based on the difference of age and eGFR level at the starting time of follow-up. Figure 3 shows the cumulative development rate of CKD based on the difference of FPG, blood pressure, and serum GGT at the starting time of follow-up. On the difference of serum GGT level, the cumulative rate of CKD at 10th year in NAFLD was 11.3% in patients with

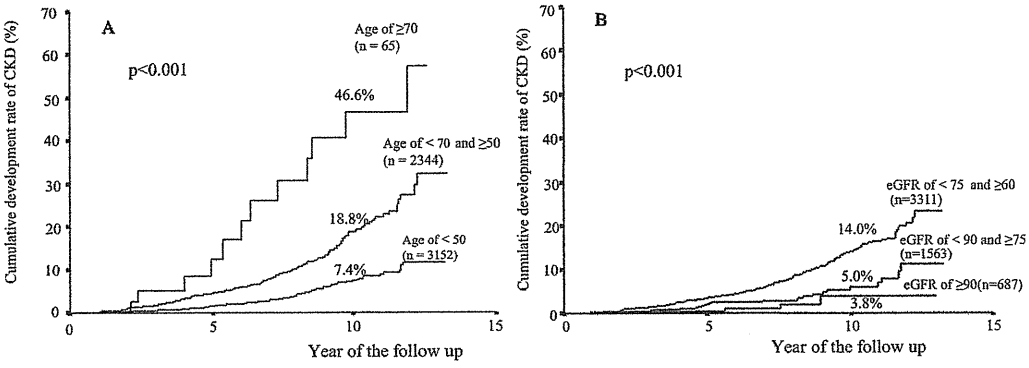

Figure 2. Cumulative development rate of CKD in NAFLD patients. Panel A: Cumulative development rate of CKD based on the difference of age at the starting time of follow-up, Panel B: Cumulative development rate of CKD based on the difference of eGFR level at the starting time of follow-up

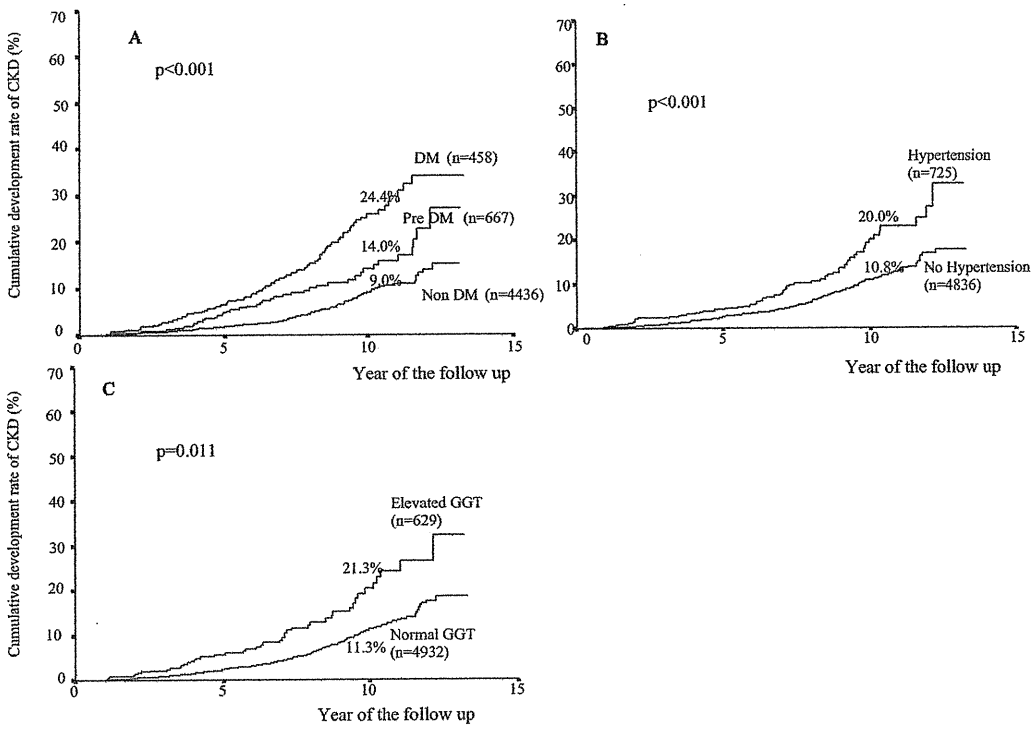

Figure 3. Cumulative development rate of CKD in NAFLD patients. Panel A: Cumulative development rate of CKD based on the difference of glucose level at the starting time of follow-up, Panel B: Cumulative development rate of CKD in patients with hypertension or without hypertension at the starting time of follow-up, Panel C: Cumulative development rate of CKD based on difference of serum GGT level at the starting time of follow-up

normal GGT level and 21.3% in those with elevated GGT level.

Impact of GGT on the incidence of CKD

In addition to elevated level of serum GGT, the four factors of ≥50 years, eGFR of 60-75 mL/min/1.73 m^2, and T2DM, hypertension were high risk factors of developing CKD with statistical significance. Figure 4 shows the cumulative development of CKD based on the difference of serum GGT in NAFLD patients with each risk factor of age of ≥50 years, eGFR of 60-75 mL/min/1.73 m^2, T2DM, or hypertension. Elevated serum GGT enhances the new development of CKD with statistically significant differences in NAFLD patients with each risk factor of ≥50 years, eGFR of 60-75 mL/min/1.73 m^2, or hypertension. In NAFLD patients with T2DM, elevated serum GGT tended to facilitate the new development of CKD (p=0.068).

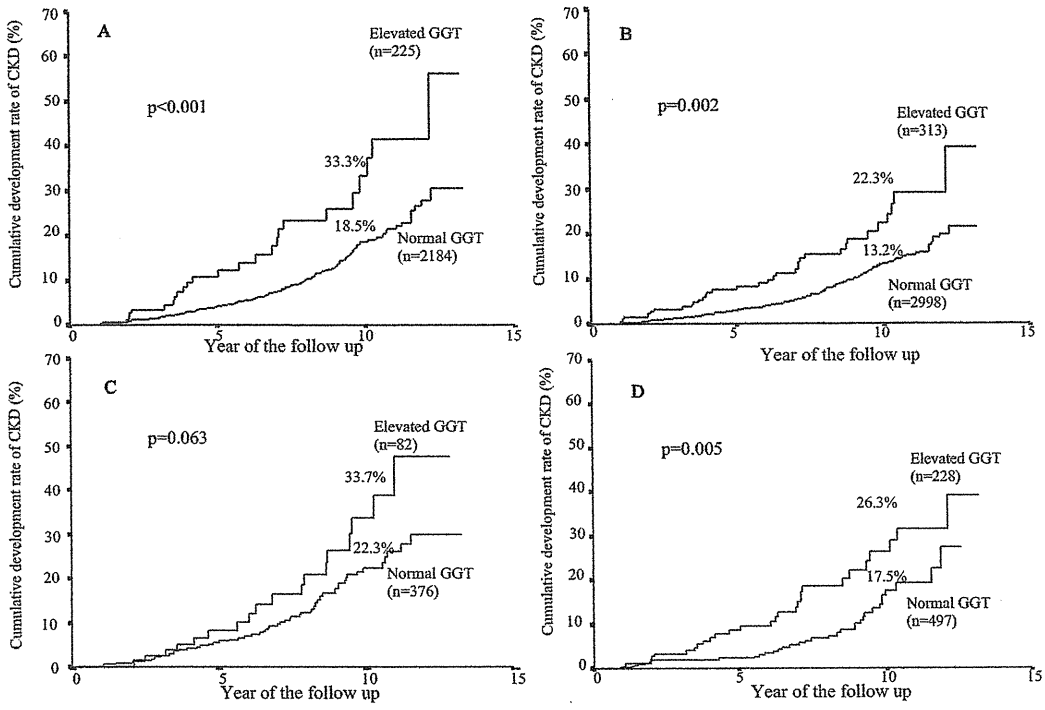

Figure 4. Cumulative development rate of CKD in NAFLD patients. Panel A: Cumulative development rate of CKD based on the difference of serum GGT level at the starting time of follow-up in NAFLD patients aged ≥50 years, Panel B: Cumulative development rate of CKD based on the difference of serum GGT level at the starting time of follow-up in NAFLD patients with eGFR of 60-75 mL/min/1.73 m^2 and absence of dipstick proteinuria (≥+1), Panel C: Cumulative development rate of CKD based on the difference of serum GGT level at the starting time of follow-up in NAFLD patients with T2DM, Panel D: Cumulative development rate of CKD based on the difference of GGT levels at the starting time of follow-up in NAFLD patients with hypertension

Discussion

We have described the incidence of development of CKD in NAFLD patients. The present study indicates that the annual incidence of CKD for a prolonged follow-up among NAFLD patients is about 1.2% based on a follow-up of 10 years. The present study was limited by a retrospective cohort trial. A blood sample and a urine sample were taken for routine analyses during follow-up. Next limitation of the study was that patients were treated with different types of exercise and diet for the NAFLD during follow-up. Moreover, although the NAFLD can be categorized into simple steatosis and steatohepatitis, the present study was undertaken without histological differentiation of simple steatosis and steatohepatitis. Next, prescribed agents during the follow-up were not considered in the present study. Finally, the interval of follow-up was different for each patient. This heterogeneity makes it slight difficult to precisely interpret the results of the study. On the other hand, the strengths of the present study are a long-term follow-up with a large numbers of patients included.

The present study shows several findings with regard to development of CKD in NAFLD patients. First, the CKD development rate in NAFLD patients with an elevated level of GGT was higher than that in those with a normal level of GGT. The fact that elevated GGT enhanced the onset of CKD is in accordance with the data reported by Chang et al (28), Ryu et al (29), and Fraser et al (30). Though the role of elevated GGT in the pathogenesis of CKD remains speculative, the following possible mechanism have been reported, 1) GGT is related to T2DM and/or insulin resistance by meta-analysis; insulin resistance may be associated with an increased risk for CKD (31-33). 2) GGT is linked with systemic low-grade inflammation; low grade inflammation may cause a change in kidney function (34). 3) GGT has been proposed as a sensitive marker of oxidative stress; oxidative stress plays an important role in renal damage (35).

Second, in addition to the elevation of GGT, the present study suggests that aging, eGFR of 60-75 mL/min/1.73 m^2, T2DM, and hypertension enhanced the development of CKD in NAFLD patients. The present findings of factors of metabolic syndrome such as T2DM and hypertension, which enhanced the new development of CKD is in accordance with the data reported by Chen et al (36), and Luk et al (37). Moreover, when GGT was elevated in NAFLD patients with each factor of ≥50 years, eGFR of 60-75 mL/min/1.73 m^2, or hypertension, the cumulative development rate of CKD increased with significant difference compared to those with a normal GGT level. In NAFLD patients with T2DM, an

Table 2. Predictive Factors for CKD Development Based on the Clinical Data at the Starting Time of Follow-up

Variables	Univariate analysis		Cox-regression	
	HR (95%CI)	p	HR (95%CI)	p
Age (years, ≥50/<50)	2.92(2.27-3.75)	< .001	2.67(2.06-3.46)	< .001
Gender (female/male)	1.08(0.73-1.60)	.706		
BMI (≥25/<25)	1.15(0.90-1.46)	.270		
Hypertension (+/-)	2.04(1.55-2.69)	< .001	1.69(1.25-2.29)	<.001
Smoking (+/-)	1.19(0.63-2.24)	.588		
AST(IU/L, ≥34/<34)	1.25(0.95-1.65)	.113		
ALT(IU/L, ≥43/<43)	1.06(0.82-1.38)	.640		
GGT (IU/L, ≥109/<109)	1.43(1.09-1.88)	.011	1.35(1.02-1.78)	.038
Diabetes (+/-)	2.42(1.85-3.17)	< .001	1.92(1.45-2.54)	< .001
WBC ($\times 10^3/mm^3$, <5.0/≥5.0)	1.04(0.80-1.35)	.770		
Hemoglobin (g/dL, <15/≥15)	1.08(0.84-1.39)	.552		
Platelet ($\times 10^4/mm^3$, <25/≥25)	1.04(0.80-1.34)	.770		
Total protein(g/dL, ≥7.5/<7.5)	0.84(0.45-1.50)	.588		
Triglyceride(mg/dL, ≥150/<150)	1.58(1.24-2.00)	< .001	1.32(0.99-1.76)	.059
Total Cholesterol (mg/dL, ≥220/<220)	1.17(0.87-1.57)	.314		
HDL Cholesterol (mg/dL, <40/≥40)	0.94(0.73-1.23)	.693		
Uric acid (mg/dL, ≥7/<7)	1.15(0.86-1.53)	.330		
eGFR (≥60 and <75 /≥75)	2.73(1.92-3.88)	< .001	2.75(1.93-3.94)	< .001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyltransferase; HDL, high density lipoprotein; HR, hazards ratio

elevated GGT indicated tendency to increase the cumulative development rate of CKD compared to those with normal GGT level.

Thus, the present results indicate that T2DM, hypertension, and elevated GGT enhance the new development of CKD in NAFLD patients. This means that in addition to the improvement of glucose level and hypertension, normalization of serum GGT could reduce the aggravation of kidney function.

NAFLD that is considered to be a risk factor for developing CKD is emerging into a new significant health problem in many countries. In addition, the life span in Japan has recently become long. In the near future, a large number of patients with NAFLD will be >60 years of age. CKD occurs more frequently in elderly patients than in young patients. Thus, it is reasonable to conclude that CKD will be increasing in NAFLD patients. CKD often progresses to ESRD with its accompanying complications. Medical physicians regarding the daily management of patients with NAFLD should check on the development of CKD in addition to the aggravation of liver function.

In conclusion, our retrospective study indicates that the annual incidence of CKD in Japanese patients with NAFLD is about 1.2%. The following five factors enhance the risk of development of CKD: low eGFR level, aging, type 2 diabetes, hypertension, and elevated GGT.

The authors state that they have no Conflict of Interest (COI).

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