Table 3 Glycated albumin/glycated hemoglobin ratio for the detection of cirrhosis (F4), severe fibrosis (F3-F4) and significant fibrosis (F2-F4) (%)

	F4	F0-F3	F3-F4	F0-F2	F2-F4	F0-F1
GA/HbA1c > 3.0	16/27 (59.3)	34/115 (29.6)	28/56 (50.0)	22/86 (25.6)	36/81 (44.4)	14/61 (23.0)
GA/HbA1c ≤ 3.0	11/27 (40.7)	81/115 (70.4)	28/56 (50.0)	64/86 (74.4)	45/81 (55.6)	47/61 (77.0)

GA/HbA1c: Glycated albumin/glycated hemoglobin.

Table 4 Aspartate aminotransferase-to-platelet ratio index for the detection of significant liver fibrosis (F2-F4)

	F2-F4 (%)	F0-F1 (%)		F2-F4 (%)	F0-F1 (%)
APRI > 0.5	68/81 (84.0)	32/61 (52.5)	APRI > 1.5	21/81 (25.9)	6/61 (9.8)
APRI ≤ 0.5	13/81 (16.0)	29/61 (47.5)	APRI ≤ 1.5	60/81 (74.1)	55/61 (90.2)

APRI: Aspartate aminotransferase-to-platelet ratio index.

Table 5 Combination of aspartate aminotransferase-to-platelet ratio index and glycated albumin/glycated hemoglobin ratio for the detection of significant liver fibrosis (F2-F4)

	F2-F4 (%)	F0-F1 (%)		F2-F4 (%)	F0-F1 (%)
APRI > 1.5 or GA/HbA1c > 3.0	43/81 (53.1)	18/61 (29.5)	APRI > 1.5 or GA/HbA1c > 3.2	34/81 (42.0)	10/61 (16.4)
Others	38/81 (46.9)	43/61 (70.5)	Others	47/81 (58.0)	51/61 (83.6)

GA/HbA1c: Glycated albumin/glycated hemoglobin; APRI: Aspartate aminotransferase-to-platelet ratio index.

been reported previously and the APRI is a simple and useful marker for the prediction of significant fibrosis. We combined the GA/HbA1c ratio and the APRI in order to examine their utility for the detection of patients with significant liver fibrosis. At first, based on prior studies[9,11,12], we assessed two cut-off points (0.50 and 1.50) of the APRI to predict the absence or presence of significant fibrosis (Table 4). When we used the cut-off point as 0.5 (Table 4; left), the sensitivity was 68/81 (84.0%) and the specificity was 29/61 (47.5%). When we used the cut-off value of 1.5 (Table 4; right), the sensitivity was 21/81 (25.9%) and the specificity was 55/61 (90.2%). Therefore, as previously reported, the cut-off point of 1.50 had a high specificity but a low sensitivity to detect significant fibrosis.

We next asked whether a combination of the GA/HbA1c and the APRI could improve the sensitivity to detect the presence of significant fibrosis and help distinguish between the two groups (F0-F1 and F2-F4). When we examined the criteria "APRI >1.5 or GA/HbA1c ratio > 3.0", the sensitivity and the specificity for the detection of significant liver fibrosis was 43/81 (53.1%) and 43/61 (70.5%), respectively (Table 5; left). In addition, when we used the criteria "APRI >1.5 or GA/HbA1c ratio > 3.2", the sensitivity was 34/81 (42.0%) and the specificity was 51/61 (83.6%) (Table 5; right). Therefore, compared with the detection of significant liver fibrosis by using the APRI alone, the combination of GA/HbA1c and the APRI (APRI >1.5 or GA/HbA1c ratio > 3.2) improved the sensitivity from 25.9% to 42.0% without a major decrease in the specificity (only a modest reduction from 90.2% to 83.6% was observed).

DISCUSSION

Liver biopsy is the gold standard method for histological evaluation of liver fibrosis[13]. Although a liver biopsy is generally a safe procedure, it is costly, invasive and has a small risk of complications. In addition, only 1/50 000 of the organ is removed and there can be sampling errors[13]. Furthermore, it has also been reported that there are inter- and intra-observer discrepancies of 10% to 20%[14,15]. Therefore, many noninvasive biomarkers readily available via laboratory tests have been proposed to predict the presence of significant fibrosis or cirrhosis in patients with HCV.

The Fibro-Test score is computed using the patient's age, sex and results of the analyses of serum haptoglobin, α2-macroglobulin, apolipoprotein A1, γ-GTP and bilirubin levels[16]. Forns *et al*[17] developed the Forns score, which is an algorithm including the platelet count, γ-GTP, age and cholesterol level. Wai *et al*[8] reported the APRI for fibrosis and cirrhosis prediction. In addition, some models such as the Hepascore[18], FibroMeter[19], FibroIndex[20] and FIB-4[21] have also been proposed for the evaluation of liver fibrosis. In addition, there are several noninvasive methods for the evaluation of liver fibrosis using ultrasound waves[22-26] such as Transient Elastography (FibroScan)[22,26]; SonoElastography (Real-Time Tissue Elastography)[23] and Acoustic Radiation Force Impulse[24-26]. Although each noninvasive tool has

an excellent positive predictive value for the diagnosis of moderate or significant fibrosis, none of the available methods completely meets the criteria of an ideal (simple, inexpensive and easily reproducible) method.

The Fibro-Test[16] is a combination of 6 markers and the Forns score[17] contains a complicated formula, indicating that while these markers are excellent, they lack simplicity. Recently introduced markers including APRI, FIB-4 and the FibroIndex are well-established, simple and inexpensive tools to assess liver fibrosis[9,20,21]. However, the values of these markers in one patient can vary within a short period, since the levels of AST or ALT or platelet count in the same patient often change daily. In addition, regarding APRI and FIB-4, the appropriate definition of the upper limit of normal (ULN) of the AST level remains uncertain, since each laboratory uses a different value for the ULN. With regard to the methods using special ultrasound tools, they are costly and cannot be routinely evaluated in all medical institutes.

In the present study, we have shown that the GA/HbA1c ratio of HCV-positive patients increases with the progression of liver fibrosis. Unlike the other previously established methods, the GA/HbA1c ratio is a simple and unique tool which is calculated based on the two glycated proteins and correlates with the degree of liver fibrosis. Since GA and HbA1c are stable over several weeks, the GA/HbA1c ratio does not change in a short period, resulting in a high reproducibility of its value. The stability of the two glycated proteins over weeks is a unique point, different from other biomarkers.

Bando *et al*[7] previously reported that the GA/HbA1c ratio in patients with CLD have an inverse correlation with the some indicators of hepatic function, regardless of the mean plasma glucose levels, thus suggesting that the increase of GA/HbA1c ratio indicates a reduction in the liver function caused by the progression of liver cirrhosis. Consistent with that report, our current histological evaluation revealed that the GA/HbA1c ratios of the cirrhotic patients were significantly higher than those of the patients without cirrhosis (Figure 2A). Furthermore, as shown in Figure 2B, the GA/HbA1c ratios increased in patients with severe fibrosis (F3-F4) compared to those in patients without severe fibrosis (F0-F2), thus suggesting that the GA/HbA1c ratio increased in correlation with the progression of fibrosis.

Since the GA/HbA1c ratio is usually about 3, we examined the diagnostic performance of the elevated GA/HbA1c ratio (GA/HbA1c > 3.0) and determined the sensitivity and specificity (Table 3). As described in the "Results" section, its solo diagnostic performance did not achieve satisfactory levels. However, when we combined the GA/HbA1c ratio with the APRI, the sensitivity to distinguish patients with significant fibrosis (F2-F4) from those without significant fibrosis was improved, with only a modest reduction in the specificity (Table 5). These findings suggest that the GA/HbA1c ratio can be used as a supportive index for the evaluation of liver fibrosis. Since only a small number of patients were investigated in the present study, we will therefore need to rigorously investigate the ratios in both larger and different populations.

In summary, we have shown that the GA/HbA1c ratio increases with the progression of the histological findings of liver fibrosis. However, its rate of change is relatively small. Although we have shown that the GA/HbA1c ratio improves the diagnostic performance of the APRI for the detection of significant fibrosis, it will be necessary to establish a new and better biomarker using a combination of the GA/HbA1c ratio and other parameter(s).

COMMENTS

Background

Hepatitis C virus (HCV) is one of the main causes of liver cirrhosis and hepatocellular carcinoma, and knowledge about the progression of liver fibrosis is important. Many noninvasive biomarkers readily available *via* laboratory tests have been proposed to predict the presence of significant fibrosis or cirrhosis in patients with HCV. The glycated albumin (GA)/glycated hemoglobin (HbA1c) ratio in patients with chronic liver disease (CLD) has been reported to show an inverse correlation with some indicators of hepatic function independent of the mean plasma glucose levels, thus suggesting that the GA/HbA1c ratio increases as the liver cirrhosis progresses. However, it has not been examined whether the GA/HbA1c ratio correlates with the histological fibrotic stage in CLD patients.

Research frontiers

Liver biopsy is the gold standard method for histological evaluation of liver fibrosis. Although a liver biopsy is generally a safe procedure, it is costly, invasive and has a small risk of complications. It is very important to establish a simple, inexpensive and easily reproducible method for the evaluation of liver fibrosis.

Innovations and breakthroughs

In the previous studies, many excellent noninvasive methods for the evaluation of liver fibrosis have been proposed. However, none of the available methods completely meets the criteria of an ideal (simple, inexpensive and easily reproducible) method. The present study has shown that the GA/HbA1c ratio of HCV-positive patients increases with the progression of liver fibrosis. Unlike the other previously established methods, the GA/HbA1c ratio is a simple and unique tool which is calculated based on the two glycated proteins and correlates with the degree of liver fibrosis.

Applications

The study showed that the GA/HbA1c ratio increased in line with the histological severity of liver fibrosis, thus suggesting that this ratio is useful as a supportive index of liver fibrosis.

Terminology

HbA1c is used as a standard index of glycemic control in patients with diabetes mellitus. Since the lifespan of erythrocytes is about 120 d, HbA1c reflects the glycemia for the recent few months; GA is another index of glycemic control which correlates with the plasma glucose levels during the past few weeks because the turnover of albumin is about 20 d.

Peer review

The study focuses on the power of the GA/HbA1c ratio in estimation of liver fibrosis in people with HCV infection. Previously defined noninvasive fibrosis markers exist but none of them have proved to be equal to liver biopsy. Therefore, research on defining new but more effective fibrosis markers should be encouraged. People with HCV are always a good research base in this context. Therefore, the present study may be interesting for the readers.

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CASE REPORT

Efficacy of pegylated interferon plus ribavirin in combination with corticosteroid for two cases of combined hepatitis C and autoimmune hepatitis

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Abstract The treatment strategy for cases of combined autoimmune hepatitis (AIH) and chronic hepatitis C (CHC) has not yet been established. A 47-year-old woman and a 53-year-old-woman were hospitalized for treatment of CHC. Ultrasonography and histological findings revealed that their liver was not cirrhotic but did have chronic damage. The histological findings of both patients were suggestive of AIH. The patients were systematically treated with pegylated interferon-alpha 2b plus ribavirin which was preceded by and combined with corticosteroid (CS), and showed sustained virological responses and normal liver function. Although these two patients with combined AIH and CHC were successfully treated with this regimen, careful attention to exacerbation of hepatic inflammation is needed because hepatitis C viral load was increased due to immunosuppression during CS treatment.

Keywords Autoimmune hepatitis · Chronic hepatitis C · Interferon · Ribavirin · Corticosteroid

Introduction

Hepatitis C virus (HCV) infection is known to be associated with various autoimmune diseases, such as autoimmune hepatitis (AIH), Sjögren's syndrome, rheumatoid arthritis and autoimmune thyroid disorders [1]. Among AIH patients, it has been reported that at least 10 % were infected with HCV in Japan [2]. Although corticosteroid (CS) therapy has been established as effective for AIH [3–5], there is concern about the possible increase in HCV caused by the immunosuppressive effect of CS in HCV-infected AIH patients. In contrast, interferon (IFN) administration, which is effective for chronic hepatitis C (CHC), has been reported to initiate acute exacerbation of AIH [6], and sometimes fulminant hepatic failure [7, 8]. Owing to these discordant treatment options for AIH and CHC, the treatment decision for patients with both of these hepatic diseases represents a dilemma.

Here we report two patients with combined AIH and CHC who showed favorable outcomes with pegylated IFN (PEG-IFN) plus ribavirin (RBV) therapy which was preceded by and combined with CS administration.

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Case reports

Case 1

A 47-year-old woman (height 153.3 cm, weight 64.5 kg) was referred to our hospital for treatment of CHC in August 2006. Although she had received IFN therapy 5 years previously, eradication of HCV had not been achieved, and her serum levels of transaminases during the therapy had been higher than baseline.

She was not a habitual drinker, and there was no history of blood transfusion, drug abuse or tattoos. There were no abnormal findings in her physical examination. Blood tests showed that the alanine aminotransferase (ALT) level was 97 IU/L, immunoglobulin (Ig) G concentration was

3457 mg/dL, anti-nuclear antibody (ANA) titer was 1:40, liver–kidney microsomal antibody-1 (LKM-1) was negative, HCV genotype was 2a and viral load was 2700 KIU/mL (Table 1). HLA typing showed DR4. To distinguish between AIH and CHC, a liver biopsy was carried out under laparoscopy. Although characteristic laparoscopic findings for AIH of multilobular and ecchymotic red macula, extensive recess, furrowed recess and rough and large tuber were not seen; microscopic findings showed considerable infiltration of plasma cells in portal areas and severe interface hepatitis, which are uncommon in CHC (Fig. 1). Therefore, we determined that the main cause of her hepatic disorder was AIH, although the diagnostic score according to the International Autoimmune Hepatitis Group (IAIHG) in 1999 [9] was 12 points, defined as 'probable' for AIH.

We started 30 mg/day prednisolone (PSL) administration in October 2006. Although the IgG level gradually decreased after initiation of PSL, the ALT level remained unchanged. Serum HCV load increased to 4800 KIU/mL during PSL administration. After 6 weeks of PSL (ALT 101 IU/L, IgG 2008 mg/dL), a weekly subcutaneous injection of 100 μg PEG-IFN-alpha-2b and daily oral administration of 800 mg RBV were started in combination with 20 mg/day PSL. The ALT level decreased gradually after starting PEG-IFN plus RBV therapy, and HCV RNA disappeared from her serum at week 8 of PEG-IFN plus RBV therapy. Subsequently, a sustained virological response (SVR) was achieved by PEG-IFN plus RBV therapy for 24 weeks. PSL was continued for 4 months after cessation of PEG-IFN plus RBV, and then withdrawn because ALT and IgG levels remained continuously normal (ALT 13 IU/L, IgG 1472 mg/dL at the end of PSL administration). From the end of the treatment to the present time, her serum ALT and IgG levels have been within the normal ranges for 3 years without any medication (Fig. 2).

Case 2

Case 2 was a 53-year-old woman (height 159 cm, weight 56.6 kg). She was diagnosed with CHC at 39 years of age, but had not taken any medication because of low serum

Table 1 Laboratory data on admission (Case 1)

WBC	4600/μL	Total protein	8.2 g/dL	IgG	3457 mg/dL
RBC	434 × 10^4/μL	Albumin	3.8 g/dL	IgA	299 mg/dL
Hb	13.1 g/dL	γ-globulin	31.9 %	IgM	47 mg/dL
Ht	39.4 %	AST	82 IU/L	ANA	40 times
Platelet	14.7 × 10^4/μL	ALT	97 IU/L	ASMA	(−)
		LDH	226 IU/L	LKM-1 Ab	(−)
		ALP	225 IU/L	AMA	(−)
		γGTP	48 IU/L	HBs Ag	(−)
		Total bilirubin	0.7 mg/dL	HBc Ab	(−)
		Cholinesterase	266 IU/L	HCV-RNA	2700 KIU/mL
				Genotype	2a
				HLA-DR	4, 9

ANA anti-nuclear antibody, *ASMA* anti-smooth muscle antibody, *LKM-1 Ab* liver–kidney microsomal antibodies type 1, *AMA* antimitochondrial antibody

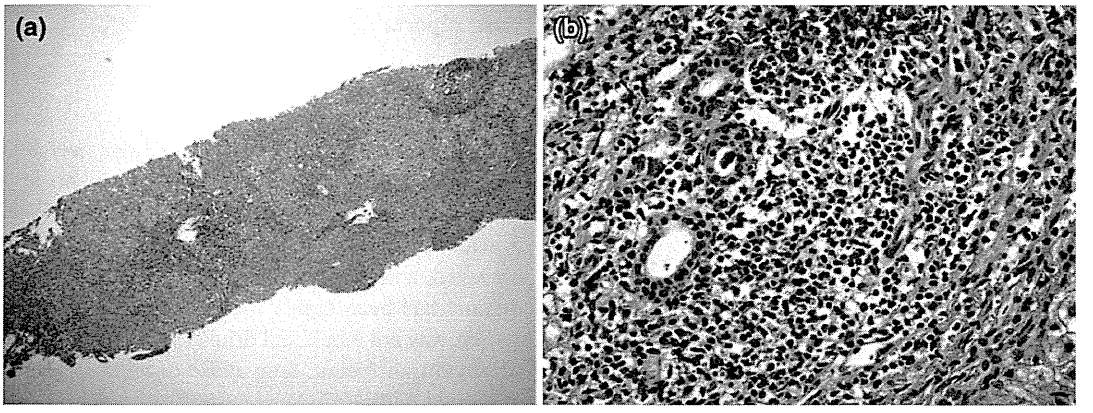


Fig. 1 Histological findings of Case 1 show considerable infiltration of plasma cells in portal areas and severe interface hepatitis. **a** H&E ×40, **b** H&E ×400

ALT levels. Symptoms of dry eye and mouth appeared in January 2008, and she was diagnosed with Sjögren's syndrome and mixed connective tissue disease based on the symptoms and serological tests, by a specialist in collagen diseases. Her hepatic function worsened after oral administration of pilocarpine hydrochloride, therefore, she was referred to our department.

She had a history of transfusion of blood coagulation factors during childbirth. Laboratory tests showed that the ALT level was 128 IU/L, IgG level was 1933 mg/dL, ANA titer was 1:1280, LKM-1 was negative, HLA typing showed DR9 and DR15, HCV genotype was 1b and viral load was 3.3 log IU/mL (Table 2). Histological findings of a liver biopsy specimen showed moderate infiltration of lymphocytes and plasma cells in portal areas, interface hepatitis and rosette formation, which are typical AIH characteristics (Fig. 3). Although the score according to the simplified criteria of AIH (IAIHG 2008) [10] was 6 points, which means 'probable' for AIH, we judged that her hepatic disorder was mainly caused by AIH, similar to Case 1.

After starting oral administration of 40 mg PSL (0.7 mg/kg) in February 2009, her ALT and IgG levels immediately decreased and became normalized. Serum HCV load increased to 5.6 log IU/mL during PSL administration. After PSL administration for 13 weeks, with a gradual decrease in dose, a weekly subcutaneous injection of 80 μg PEG-IFN-alpha-2b and daily oral 600 mg RBV were started in combination with 20 mg/day PSL. HCV RNA disappeared from her serum at week 8 of PEG-IFN plus RBV therapy, and an SVR was achieved by continuing the treatment for 48 weeks. After the end of the PEG-IFN plus RBV therapy, PSL dose was gradually decreased and

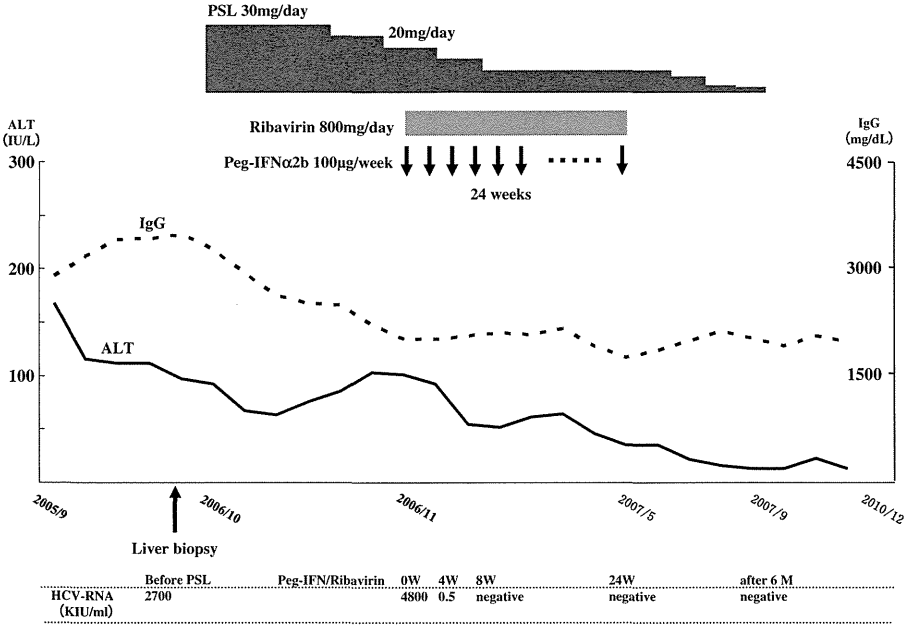


Fig. 2 Clinical course of Case 1

Table 2 Laboratory data on admission (Case 2)

WBC	3600/μL	TP	7.3 g/dL	IgG	1933 mg/dL
RBC	463 × 10^4/μL	Albumin	3.9 g/dL	IgA	322 mg/dL
Hb	13.5 g/dL	γ-globulin	25.5 %	IgM	156 mg/dL
Ht	40.4 %	AST	108 IU/L	ANA	1280 times
Platelet	15.1 × 10^4/μL	ALT	128 IU/L	ASMA	(−)
		LDH	269 IU/L	LKM-1 Ab	(−)
		ALP	169 IU/L	AMA	(−)
		γGTP	85 IU/L	HBs Ag	(−)
		Total bilirubin	1.3 mg/dL	HBc Ab	(+)
		Cholinesterase	290 IU/L	HBV-DNA	(−)
				HCV-RNA	3.3 log IU/mL
				Genotype	1b
				HLA-DR	9, 15

ANA anti-nuclear antibody, *ASMA* anti-smooth muscle antibody, *LKM-1 Ab* liver–kidney microsomal antibodies type 1, *AMA* antimitochondrial antibody

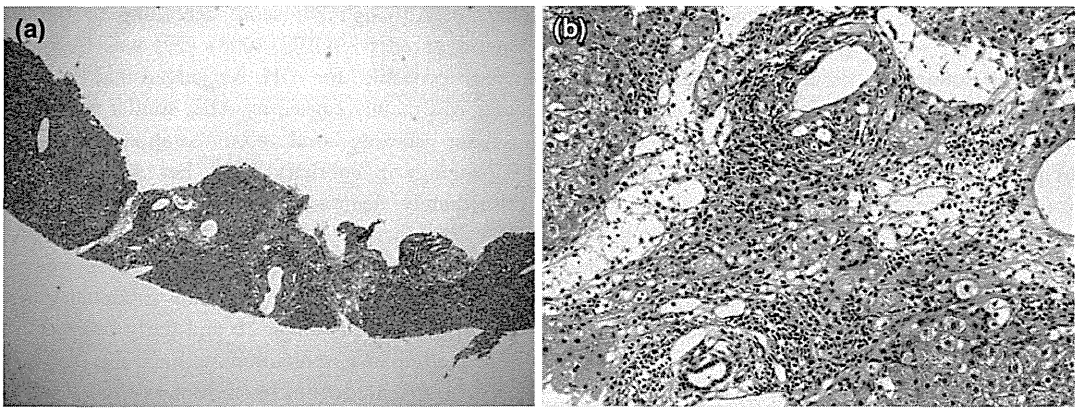
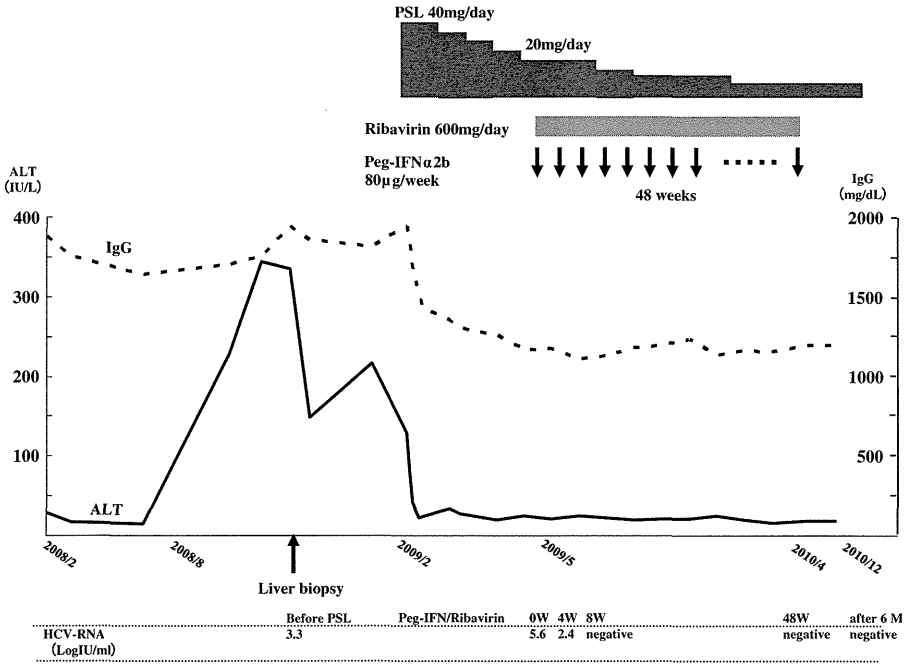


Fig. 3 Histological findings of Case 2 show moderate infiltration of lymphocytes and plasma cells in portal areas, interface hepatitis and rosette formation. **a** H&E ×40, **b** H&E ×400

Fig. 4 Clinical course of Case 2



daily administration of 5 mg has continued to date. Consequently, her ALT and IgG levels have remained within the normal range (Fig. 4).

Discussion

We have reported two patients with features of AIH together with HCV infection, who were successfully treated with PEG-IFN plus RBV therapy preceded by and combined with steroid administration. This therapeutic challenge may represent one approach for hepatitis in patients with combined AIH and CHC.

The most important issue in this approach is how to judge whether the autoimmunity is associated with hepatic inflammation in patients with HCV infection.

CHC patients sometimes become positive for autoantibodies such as ANA, therefore, it is difficult to distinguish serologically between simple CHC and CHC combined with AIH. A variety of type 2 AIH, which is characterized by anti-LKM-1 antibodies in the serum, has been reported with HCV-associated AIH [11]. However, the positivity rate of anti-LKM-1 antibodies in Japanese CHC patients is low [12], and our two cases were actually both negative.

There are some reports indicating the importance of histological manifestations such as severe piecemeal

necrosis, lobular hepatitis, multinucleated giant cells, and moderate or severe infiltration of plasma cells, which are microscopic characteristics of AIH, to distinguish CHC accompanied with AIH from simple CHC [13].

We diagnosed CHC combined with AIH based on changes in the level of ALT during previous IFN treatment and histological findings in Case 1, and on other accompanying autoimmune diseases and histological findings in Case 2.

However, the treatment strategy for combined AIH/HCV has not yet been established. It is known that IFN often induces acute exacerbation of AIH, and occasionally fulminant hepatic failure [6–8], therefore, many reports have recommended CS therapy for these patients [14, 15]. In contrast, there are some reports showing that IFN therapy is more effective than CS, even in combined AIH/CHC [16]. Petersen-Benz et al. [17] reported successful treatment of a case with AIH/CHC overlap syndrome. First, they treated AIH with CS for several years, and then switched to IFN plus RBV therapy for CHC, and achieved HCV eradication. However, readministration of CS was required to inhibit hepatic inflammation in this case. Therefore, we planned pretreatment with CS and subsequent IFN plus RBV therapy combined with continued CS for our two cases. PEG-IFN plus RBV therapy was started at 6 weeks of CS treatment in Case 1 versus at 13 weeks in Case 2 because the ALT level did not decrease steadily with CS administration in Case 1. As a result, favorable viral eradication was achieved without aggravation of hepatic inflammation in both cases.

Careful attention to viral breakthrough caused by the immunosuppressive effect of CS is required. Indeed, HCV load increased during CS administration in both of our cases. Therefore, when this therapeutic regimen is administered, it is necessary to monitor the ALT level closely until start of antiviral therapy, so as not to miss any exacerbation of hepatic inflammation.

Judging from the changes in IgG and ALT levels during CS treatment, it is speculated that the hepatic inflammation in Case 1 was caused by both HCV and autoimmunity, whereas that in Case 2 was mainly caused by AIH. Therefore, after termination of IFN plus RBV therapy, we attempted to stop CS treatment in Case 1, but continued a low dose PSL in Case 2.

In conclusion, although antiviral therapy combined with CS needs to be carefully applied, it may represent a worthwhile treatment for CHC patients with clinical and histological characteristics of AIH.

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

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Prevalence and associated metabolic factors of nonalcoholic fatty liver disease in the general population from 2009 to 2010 in Japan: a multicenter large retrospective study

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Abstract
Background The prevalence of nonalcoholic fatty liver disease (NAFLD) has been increasing. This study aimed to assess the recent prevalence of NAFLD and to predict the prevalence of nonalcoholic steatohepatitis (NASH) with liver fibrosis using established scoring systems in the general population.
Methods A cross-sectional study was conducted among 8352 subjects who received health checkups from 2009 to 2010 in three health centers in Japan. Subjects with an intake over 20 g of alcohol/day or with other chronic liver diseases were excluded. Fatty liver was detected by ultrasonography. The probability of NASH with advanced fibrosis was calculated according to the body mass index, age, ALT, and triglyceride (BAAT) and FIB-4 (based on age, aspartate aminotransferase and alanine aminotransferase levels, and platelet counts) indices.
Results A total of 5075 subjects were enrolled. The overall prevalence of NAFLD was 29.7%. There was a significant threefold difference in the mean prevalence between males (41.0%) and females (17.7%). This prevalence showed a linear increase with body mass index, triglycerides, and low-density lipoprotein cholesterol regardless of threshold values, even without obesity. The estimated prevalence of NASH according to the BAAT index ≥ 3 was 2.7%, and according to the FIB-4 index it was 1.9%.
Conclusions The prevalence of NAFLD has increased in the general population, especially in males. There is a linear relationship between the prevalence of NAFLD and various metabolic parameters, even in nonobese subjects. The prevalence of NASH with advanced fibrosis is estimated to be considerably high in subjects with NAFLD.

Keywords Abdominal obesity · Central obesity · Metabolic syndrome

Abbreviations

Ht	Body height
BW	Body weight
BMI	Body mass index
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
AAR	AST/ALT ratio
ALP	Alkaline phosphatase
GGT	Gamma-glutamyl transferase
ChE	Cholinesterase
FPG	Fasting plasma glucose

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Hb	Hemoglobin
PLT	Platelet
TC	Total cholesterol
TG	Triglyceride
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol

Introduction

Obesity and life-related diseases due to obesity are rising at an alarming rate in Japan, many Western countries, and worldwide. Nonalcoholic fatty liver disease (NAFLD), a hepatic manifestation of metabolic syndrome, is associated with an increased risk for development of life-related disease including type 2 diabetes, cardiovascular disease, and cerebral vessel disease. NAFLD covers a spectrum of liver diseases that range from benign simple steatosis to hepatic inflammation and fibrosis of nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma [1, 2].

NAFLD is rapidly becoming the most common liver disorder worldwide [3–6]. Currently, NAFLD is present in 20–40% the general population of industrialized countries [7, 8]. Among all subjects with NAFLD, features of NASH are observed in 10–20%. Recent studies reported that the prevalence of NASH in Western countries is approximately 2–12% [7–9].

The degree of fatty infiltration in NAFLD is graded according to the percentage of hepatocytes with fat deposits: mild NAFLD involves less than 30% hepatocytes, moderate NAFLD up to 60%, and severe NAFLD more than 60%. The degree of liver fibrosis must be estimated to determine the surveillance, prognosis, and optimal treatment for NAFLD, similar to the situation for other liver diseases such as chronic hepatitis C [10, 11]. Liver biopsy is recommended as the gold standard method for the diagnosis and staging of NAFLD/NASH, but it is invasive and is associated with a high risk of complications [1, 12]. In fact, it is impossible to recommend a liver biopsy to all NAFLD patients, because the number of NAFLD patients has reached 80–100 million in the USA and an estimated 10 million in Japan. Previous studies proposed novel scoring systems to estimate NASH with advanced liver fibrosis, because it was just not realistic to conduct a liver biopsy in a large number of subjects with fatty liver. The scoring system consisting of body mass index (BMI), age, serum alanine aminotransferase (ALT), and triglyceride (BAAT score) and the novel index proposed by Sterling et al. based on age, serum aspartate aminotransferase (AST), ALT level, and platelets (FIB-4 index) are simple and useful to predict NASH with advanced liver fibrosis. It might therefore be possible to estimate the approximate prevalence of NASH with advanced liver fibrosis in the Japanese general population by using these predictive formulae [13–15].

It is well known that there are age and gender differences in both the prevalence and severity of NAFLD. These age and gender differences are caused by differences in the prevalence of obesity and lifestyle-related diseases. According to annual health check findings in Japan, the prevalence of NAFLD in men is approximately 27% for all ages above 30 years. In contrast, in women, it gradually increases from 7% in their 30s to 23% above 60 years of age [16, 17]. However, this information was reported from studies conducted at the end of 1990–2000. According to the worldwide systemic analysis of health examination surveys and epidemiological studies, the prevalence of obesity is increasing year-on-year and varies substantially between nations. It is predicted that the prevalence of NAFLD in the general population is increasing and there might be a difference between each country [18].

Because of the dramatic increase in obesity in Japan and many other industrialized countries, it is plausible that there also has been a dramatic increase in the prevalence of NAFLD and NASH. However, the most recent prevalence of NAFLD has not been well established in Japan. Therefore, the aim of this study was to investigate the prevalence of NAFLD/NASH using the latest database of a large proportion of the general population who underwent an annual health checkup from 2009 to 2010 in Japan and to estimate the prevalence of NASH with liver fibrosis using established scoring systems.

Patients and methods

Study population

We studied 8352 subjects (51.8% males) aged 21–86 years (mean 50.0 years), who received a health checkup from 2009 to 2010 in three health centers, namely Eguchi Hospital Health Center in Saga prefecture, Kawamura Clinic Heath Center in Hiroshima prefecture, and Kochi Medical School Hospital in Kochi prefecture in Japan. Subjects were included if they fulfilled the following criteria: (1) absence of markers of hepatitis B virus infection (hepatitis B surface antigen and anti-hepatitis B core antibody) and hepatitis C virus infection (anti-hepatitis C virus antibodies); (2) no alcoholic liver disease (more than 20 g of alcohol per day); and (3) no use of insulin-sensitizing medication. Finally, 5075 subjects who met the inclusion criteria were enrolled. All subjects provided written informed consent to the use of their data for an epidemiological study under anonymity. The study design

was approved by each institutional review board (Saga Medical School, "2011-06-04" as Eguchi Hospital; Hiroshima University, "Eki-241" as Kawamura Clinic Health Center; Kochi Medical School, "23-74"). The study was conducted in accordance with the Declaration of Helsinki.

Physical examination and serum biochemistry

Body weight and height were obtained for both sets of subjects, and BMI was calculated. Waist circumference was measured at the umbilical level. Venous blood samples were taken from all subjects at 0900 hours following a 12-h overnight fast and AST, ALT, gamma-glutamyl transpeptidase (GGT), total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and fasting plasma glucose (FPG) levels were measured using standard techniques in the subjects who received a health checkup.

Abdominal ultrasound protocol and definition of fatty liver

All subjects received abdominal ultrasonography to determine fatty liver. First, examination of all visible liver parenchyma was performed with a conventional convex array transducer. Liver parenchyma was examined with sagittal as well as longitudinal guidance of a probe and completed by lateral and intercostals views. Use of tissue harmonic imaging with both transducers was encouraged. The presence of steatosis was recognized as a marked increase in hepatic echogenicity, poor penetration of the posterior segment of the right lobe of the liver, and poor or no visualization of the hepatic vessels and diaphragm. The severity of hepatic steatosis present by imaging was not graded with careful consideration of the error due to the difference of ultrasonography equipment and examiner. The liver was considered normal if the hepatic parenchyma was homogeneous with no acoustic attenuation, the portal veins were visible, the diaphragm was well visualized, and the echogenicity was similar or slightly higher than the echogenicity of the renal cortex.

Ultrasonography was performed with the following units: LOGIQ 7 with a 4-MHz convex array transducer (GE Health Care) at Eguchi Hospital; Pro Sound Alpha-10 with 3.5 MHz with a convex array transducer (Hitachi Aloka Medical) at Kawamura Clinic Health Center; and Xario with a 3.5-MHz convex array transducer (Toshiba Medical Systems) at Kochi Medical School. Experienced sonographers, who were trained by gastroenterologists with more than 5 years' experience, performed examinations over 5 years. Technical parameters were adjusted for each subject using the standard protocol for ultrasonography. Each certificated gastroenterologist independently reviewed the images and evaluated the liver for the presence of steatosis.

Algorithms for prediction of NASH

In this study, two representative algorithms based on the BAAT score and the recently proposed FIB-4 index [13, 14] were employed to predict the prevalence of subjects with NASH with advanced liver fibrosis. BAAT scores consist of the sum of the following categorical variables: BMI ($\geq$28 = 1, <28 = 0), age ($\geq$50 years = 1, <50 = 0), ALT [$\geq$2 UNL (males, ALT $\geq$60 IU/L; females, ALT $\geq$40 IU/L) = 1, <2 UNL = 0) and serum triglycerides [1.7 mmol/L (=150 mg/dL) = 1, <1.7 = 0], thus ranging from 0 to 4, and a cutoff value to predict NASH with advanced liver fibrosis was defined as BAAT score $\geq$3 in this study [13]. The FIB-4 index was calculated as [age (years) $\times$ AST (U/L)]/[platelets (10^9) $\times$ root ALT (U/L)]. The subjects were classified into three groups on the basis of the following values: FIB-4 index $\geq$2.67 and <1.30, because previous studies reported that a FIB-4 index $\geq$2.67 had an 80% positive predictive value and a FIB-4 index <1.30 had a 90% negative predictive value to predict NASH with advanced liver fibrosis [14, 15].

Statistical analysis

Descriptive statistics (means and standard deviations) were calculated for all continuous variables. Differences between the two groups were compared by the Mann–Whitney U test. Differences were considered significant at $p < 0.05$. All analyses were carried out using IBM SPSS Statistics Ver. 19.

Results

Clinical and biochemical characteristics and the prevalence of NAFLD in enrolled subjects

A total of 5075 subjects were enrolled from July 2009 to June 2010. The clinical and biochemical characteristics of these subjects are summarized in Tables 1 and 2. The subjects were predominantly middle-aged (50.0 $\pm$ 9.5 years; range 21–86 years) and 48.2% were female. The mean BMI of the whole cohort was 23.0 $\pm$ 3.3 kg/m^2 with 23.6% of the subjects meeting the criteria for obesity (BMI $\geq$25). The mean age was not significantly different between subjects with or without NAFLD (51.1 $\pm$ 8.9 vs. 49.5 $\pm$ 6.7 years). A total of 1509 subjects (29.7%) had evidence of NAFLD on ultrasonography. There was a significant threefold difference in the mean prevalence of NAFLD between males (41.0%) and females (17.7%). The

Table 1 Characteristics of all patients

	All ($n = 5075$)	Non-NAFLD ($n = 3566$)	NAFLD ($n = 1509$)	p value
Gender (M/F)	2627/2448	1551/2015	1076/433	<0.0001
Age (years)	50.0 ± 9.5	49.5 ± 6.7	51.1 ± 8.9	<0.0001
Ht (m)	1.631 ± 0.086	1.62 ± 0.08	1.65 ± 0.08	<0.0001
BW (kg)	61.4 ± 11.7	57.7 ± 9.6	70.2 ± 11.3	<0.0001
BMI (kg/m^2)	23.0 ± 3.3	21.8 ± 2.6	25.6 ± 3.3	<0.0001
AST (IU/L)	21.5 ± 9.1	20.1 ± 6.7	24.7 ± 12.4	<0.0001
ALT (IU/L)	22.6 ± 16.6	18.3 ± 9.9	32.7 ± 23.5	<0.0001
AAR	1.11 ± 0.37	1.21 ± 0.35	0.87 ± 0.29	<0.0001
ALP (IU/L)	210.6 ± 65.7	205.3 ± 65.3	223.3 ± 64.7	<0.0001
GGT (IU/L)	34.4 ± 36.1	28.4 ± 27.0	48.5 ± 48.6	<0.0001
ChE (IU/L)	293.6 ± 126.0	277.0 ± 122.0	33.4 ± 126.3	<0.0001
Albumin (g/dL)	4.5 ± 0.2	4.5 ± 0.2	4.6 ± 0.2	<0.0001
FPG (mg/dL)	99.6 ± 17.7	96.3 ± 13.3	107.3 ± 23.3	<0.0001
TC (mg/dL)	207.1 ± 34.1	205.6 ± 33.9	210.5 ± 34.3	<0.0001
TG (mg/dL)	111.8 ± 76.9	93.0 ± 52.6	155.6 ± 102.3	<0.0001
HDL-C (mg/dL)	60.6 ± 16.2	64.4 ± 16.2	51.5 ± 12.1	<0.0001
LDL-C (mg/dL)	121.6 ± 32.1	118.3 ± 32.2	129.4 ± 30.6	<0.0001

Values are expressed as mean ± SD. Statistical analysis was conducted using Mann–Whitney U test

Ht body height, *BW* body weight, *BMI* body mass index, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *AAR* AST/ALT ratio, *ALP* alkaline phosphatase, *GGT* gamma-glutamyl transferase, *ChE* cholinesterase, *FPG* fasting plasma glucose, *Hb* hemoglobin, *PLT* platelet, *TC* total cholesterol, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

prevalence of NAFLD in men was greater than 30% in all ages above the third decade. This prevalence was higher in males than that in females for all ages, and it gradually increased from only 3.3% in the second decade to 31.3% above the sixth decade in females (Fig. 1). Furthermore, there was a significant difference in most of the clinical factors except BMI and LDL cholesterol between males and females (Table 2). By multivariate logistic regression in each male and female with NAFLD, there was a difference in the independent variables significantly associated with NAFLD (Table 3).

Relationship between anthropometric and biochemical features and the presence of NAFLD

BMI in subjects with NAFLD was significantly higher than that in those without NAFLD ($p < 0.01$). The prevalence of NAFLD showed a linear increase with the increase of BMI (BMI <23 kg/m^2, 10.5%; BMI ≥23 kg/m^2 and <25 kg/m^2, 37.9%; BMI ≥25 kg/m^2 and <28 kg/m^2, 58.4%; BMI ≥28 kg/m^2, 84.2%; a 7.4–11.4% increase per 1 kg/m^2 between 20 and 30) (Fig. 2), and it was 18.4% in nonobese subjects (BMI <25 kg/m^2) with NAFLD, 63.4% in obese subjects (BMI >25 kg/m^2 but <30 kg/m^2) with NAFLD, and 89.1% in morbid obese (BMI >30 kg/m^2) subjects with NAFLD. Serum levels of LDL cholesterol, triglycerides (TG), FPG, and liver enzymes including AST and ALT were significantly higher in subjects with NAFLD than those in subjects without NAFLD ($p < 0.01$). Serum levels of HDL cholesterol were significantly lower in subjects with NAFLD than those without NAFLD ($p < 0.01$, Tables 1, 2). The prevalence of NAFLD showed a linear increase with the increase of serum triglycerides and LDL cholesterol levels (Fig. 3a, d), and a linear decrease with the increase of serum HDL cholesterol levels (Fig. 3c). The prevalence of NAFLD was 22.8% in subjects with normal triglyceride levels (triglycerides <150 mg/dL) and 59.5% in subjects with hypertriglyceridemia (triglycerides >150 mg/dL). The prevalence of NAFLD was 27.3% in subjects with normal HDL cholesterol levels (HDL cholesterol >40 mg/dL) and 61.7% in subjects with hypo-HDL cholesteremia (HDL cholesterol <40 mg/dL). The prevalence of NAFLD was 26.4% in subjects with normal LDL cholesterol levels (LDL cholesterol <140 mg/dL) and 38.5% in subjects with hyper-LDL cholesteremia (LDL cholesterol >140 mg/dL). The prevalence of NAFLD showed a linear increase with FPG levels (<120 mg/dL) and this prevalence was approximately 60% and reached a plateau with FPG ≥120 mg/dL, especially in males (Fig. 3e). The prevalence of NAFLD was 25.6% in subjects with a normal fasting glucose, 56.2% in subjects with impaired FPG (FPG >110 mg/dL

Table 2 Characteristics of the patients according to gender

	Male			Female			
	Non-NAFLD (n = 1551)	NAFLD (n = 1076)	p value*	Non-NAFLD (n = 2015)	NAFLD (n = 433)	p value*	p value**
Age (years)	49.8 ± 10.2	49.9 ± 8.8	0.651	49.2 ± 9.3	54.0 ± 8.3	<0.0001	<0.0001
Ht (m)	1.69 ± 0.58	1.694 ± 0.059	0.886	1.567 ± 0.055	1.552 ± 0.055	<0.0001	<0.0001
BW (kg)	64.8 ± 7.9	73.6 ± 10.3	<0.0001	52.2 ± 6.8	61.8 ± 9.2	<0.0001	<0.0001
BMI (kg/m^2)	22.6 ± 2.4	25.6 ± 3.1	<0.0001	21.2 ± 2.6	25.7 ± 3.7	<0.0001	0.5978
AST (IU/L)	20.9 ± 7.8	25.2 ± 11.7	<0.0001	19.5 ± 5.7	23.5 ± 14.0	<0.0001	<0.0001
ALT (IU/L)	21.1 ± 11.7	35.3 ± 24.0	<0.0001	16.1 ± 7.5	26.2 ± 20.6	<0.0001	<0.0001
AAR	1.09 ± 0.34	0.81 ± 0.26	<0.0001	1.30 ± 0.32	1.01 ± 0.30	<0.0001	<0.0001
ALP (IU/l)	214.3 ± 63.9	218.6 ± 58.5	<0.01	198.1 ± 65.6	235.4 ± 77.3	<0.0001	<0.01
GGT(IU/L)	36.5 ± 31.5	53.8 ± 48.7	<0.0001	22.1 ± 20.9	35.4 ± 45.9	<0.0001	<0.0001
ChE (IU/L)	289.2 ± 120.5	333.2 ± 130.0	<0.0001	267.2 ± 122.3	333.8 ± 116.9	<0.0001	<0.05
Albumin (g/dL)	4.5 ± 0.2	4.6 ± 0.2	<0.0001	4.4 ± 0.2	4.5 ± 0.2	<0.0001	<0.0001
FPG (mg/dL)	99.7 ± 15.2	108.7 ± 23.8	<0.0001	93.7 ± 11.0	103.9 ± 21.5	<0.0001	<0.0001
TC (mg/dL)	200.7 ± 32.1	208.0 ± 33.9	<0.0001	209.4 ± 34.7	216.6 ± 34.7	<0.0001	<0.0001
TG (mg/dL)	109.6 ± 59.1	167.2 ± 106.7	<0.0001	80.2 ± 42.9	127.0 ± 84.1	<0.0001	<0.0001
HDL-C (mg/dL)	57.5 ± 14.5	48.6 ± 10.4	<0.0001	69.8 ± 15.4	58.6 ± 13.0	<0.0001	<0.0001
LDL-C (mg/dL)	118.5 ± 31.8	128.6 ± 30.5	<0.0001	118.1 ± 32.5	131.3 ± 30.8	<0.0001	0.1288

Values are expressed as mean ± SD. Statistical analysis was conducted using Mann–Whitney U test. Abbreviations are the same as those in Table 1

*p value for comparison between non-NAFLD and NAFLD in each gender group

**p value for comparison between male and female with NAFLD

Fig. 1 Prevalence of NAFLD in patients according to age. The prevalence of NAFLD is higher in males than that in females at all ages, and it gradually increases with age in females

but <126 mg/dL), and 68.0% in subjects with FPG greater than 126 mg/dL, respectively. The prevalence of NAFLD gradually increased with an elevation of ALT. The prevalence of NAFLD was 70.6 and 35.8% in subjects with abnormal ALT levels in males (ALT ≥30) and females (ALT ≥20); the prevalence of NAFLD was 29.5 and 10.7% in subjects with normal ALT levels in males (ALT <30) and females (ALT <20), respectively (Fig. 3f).

Prevalence of NASH in the general population and subjects with fatty liver predicted by established scoring systems

In this study, the prevalence of NASH was estimated by BAAT score and FIB-4 index. Tables 4 and 5 show the distribution of subjects estimated by BAAT score and FIB-4 index, respectively. The estimated prevalence of NASH according to the BAAT index was 16.7% (BAAT score ≥2) and 2.7% (BAAT score ≥3) in the whole cohort, whereas it was 36.1% (BAAT score ≥2) and 8.3% (BAAT score ≥3) in subjects with NAFLD.

Mean FIB-4 indices in the whole cohort, in subjects without NAFLD, and in those with NAFLD were 1.15 ± 0.60, 1.17 ± 0.62, and 1.10 ± 0.55, respectively. The estimated prevalence of NASH according to the FIB-4 index was 1.9% (cutoff ≥2.67) in the whole cohort and it was 2.7% in subjects with NAFLD. In contrast, the estimated prevalence of NAFLD without advanced fibrosis was 74.0% (cutoff <1.30) in subjects with NAFLD.

Discussion

Using the latest large database in Japan, our study showed that the prevalence of NAFLD was high in the general

Table 3 Clinical factors associated with NAFLD in each male and female group using multivariate logistic regression analysis

	Male				Female			
	Coefficient	*p*	Odds ratio	95% confidence interval	Coefficient	*p*	Odds ratio	95% confidence interval
BMI >25	1.34	0.00	3.81	3.11–4.67	1.98	0.00	7.23	5.50–9.50
Age >50	–	–	–	–	0.59	0.00	1.80	1.37–2.37
ALT (M >30; F >20)	0.93	0.00	2.54	2.00–3.23	0.50	0.00	1.65	1.20–2.67
AAR <1	1.08	0.00	2.53	2.02–3.15	1.07	0.00	2.56	1.87–3.48
FPG >110	0.851	0.00	2.34	1.83–2.99	0.96	0.00	2.61	1.76–3.87
TG >150	0.792	0.00	2.21	1.78–2.74	0.95	0.00	2.58	1.83–3.63
GGT >35	0.360	0.00	1.43	1.17–1.75	–	–	–	–
HDL <40	0.306	<0.05	1.36	1.00–1.84	–	–	–	–
LDL >140	0.264	<0.05	1.30	1.05–1.60				

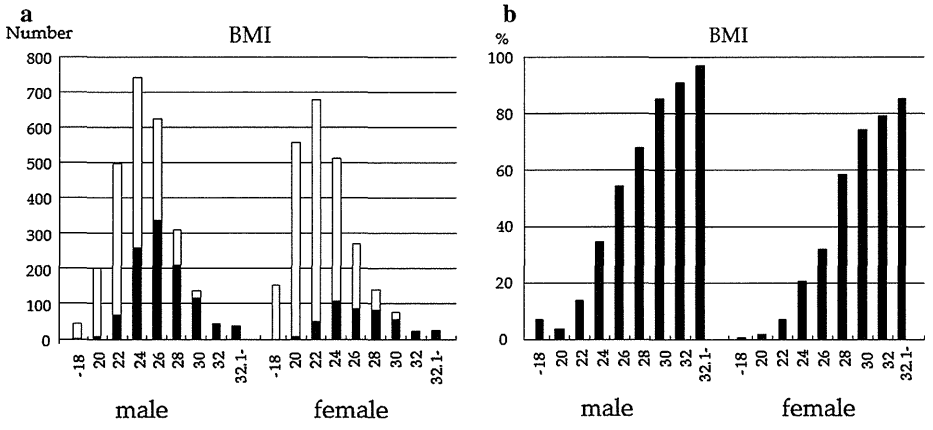

Fig. 2 **a** Distribution of subjects with NAFLD (*black columns*) and without NAFLD (*white columns*) according to body mass index (BMI). **b** Relative percentage of NAFLD according to BMI. The prevalence of NAFLD shows a tendency to increase linearly with BMI in males and females

population, especially in males, even though subjects were not obese. Our study suggested that the prevalence of NAFLD is still increasing in Japan. The present study showed the most recent frequency of NAFLD and a 10% increase from a previous Japanese study conducted in subjects who received a health checkup from 1989 to 2000 [17]. A recent study reported that mean BMI has globally increased in adults 20 years and older in 199 countries and territories between 1980 and 2008 [18]. In 2008, an estimated 1.46 billion adults worldwide had a BMI of 25 kg/m^2 or greater, and of these, 205 million men and 297 million women were obese.

Our study found that there was a linear relationship between the prevalence of NAFLD and an increase in BMI, serum triglycerides, and cholesterol, whereas the increase of prevalence showed a plateau at 120 mg/dL for FPG levels, especially in males. It is well known that NAFLD and NASH are strongly associated with the presence of obesity and lifestyle-related diseases, especially type 2 diabetes mellitus [6–8]. According to annual health check findings in Japan and Asian countries, the prevalence of NAFLD increases with BMI; it has been reported to be 10–20% in nonobese subjects, approximately 50% in those with a BMI ranging from more than 25 kg/m^2 to less than 30 kg/m^2, and approximately 80% in those with a BMI over 30 kg/m^2 [19].

A previous study reported that the crude prevalence of NAFLD increased with deterioration of glucose homeostasis, from 27% in patients with normal fasting glucose, 43% in patients with impaired fasting glucose, and 62% in patients with newly diagnosed and thus untreated diabetes [20]. This study revealed that there were a certain number of NAFLD subjects with normal range in various parameters. It is unclear whether NAFLD causes metabolic dysfunction or whether metabolic dysfunction is responsible for hepatic fat accumulation, or both. As shown in our study, there was a close relationship between the pathogenesis of NAFLD and that of glucose and lipid metabolism abnormalities.

Our study confirmed previous findings that various traditional metabolic parameters and aminotransferases may be normal in an appreciable proportion of patients with NAFLD, and therefore, are not sensitive enough for the diagnosis of NAFLD [7, 21–23]. It is widely accepted that

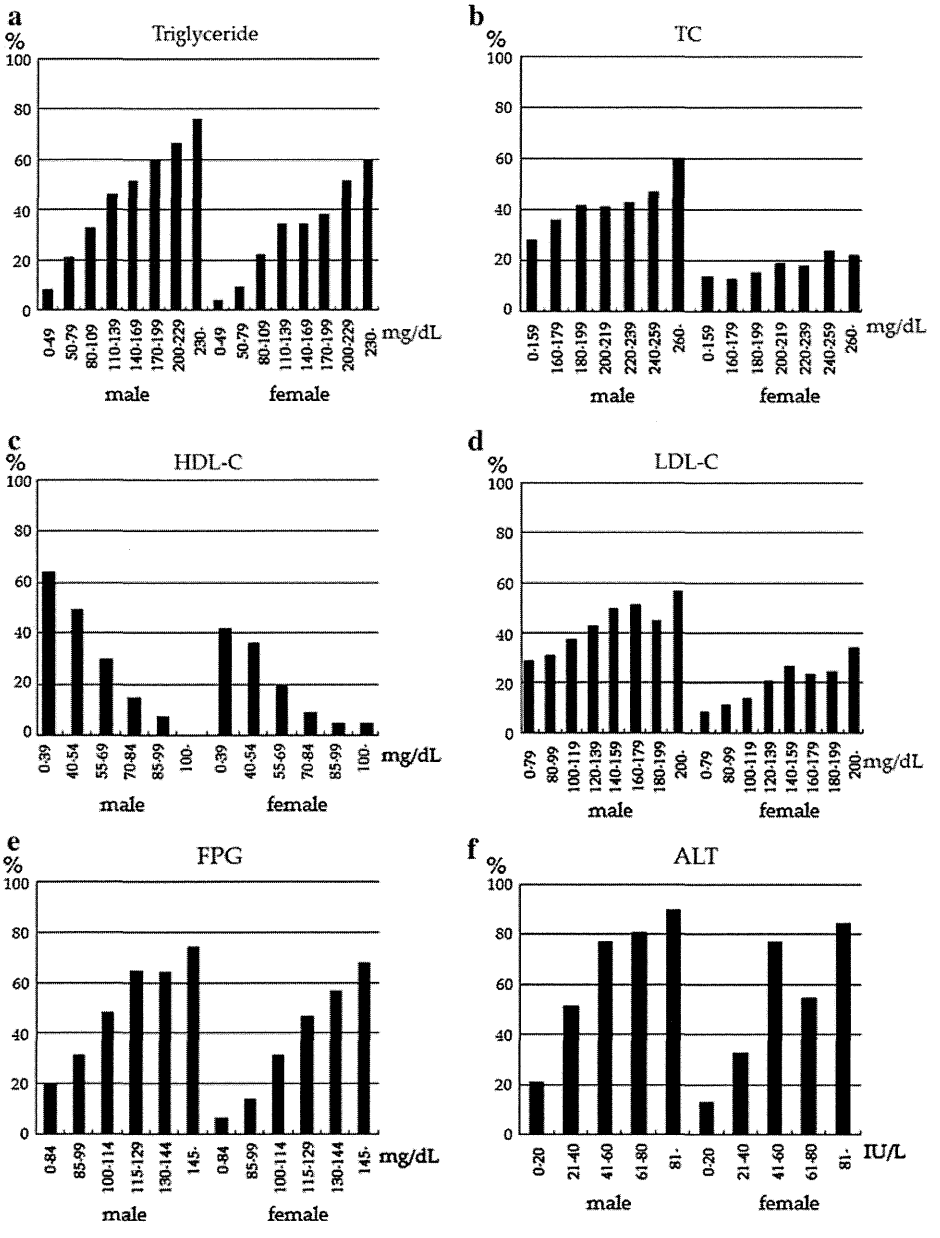

Fig. 3 Prevalence of NAFLD in various variables. The prevalence of NAFLD shows a linear increase with serum levels of triglycerides and low-density lipoprotein cholesterol (LDL-C) (**a**, **d**), whereas there is a linear decrease with high-density lipoprotein cholesterol (HDL-C) (**c**). The prevalence of NAFLD shows a linear increase with fasting plasma glucose (FPG) (<120 mg/dL), and this prevalence is approximately 60% and reaches a plateau with glucose ≥120 mg/dL, especially in males (**e**). The prevalence of NAFLD gradually increases with the elevation of alanine aminotransferase (ALT). There is an appreciable amount of NAFLD subjects with normal ALT levels (**f**)

Table 4 Distribution of subjects according to BAAT score

	BAAT score					Total
	0	1	2	3	4	
Non-NAFLD	1555	1708	290	13	0	3566
NAFLD	277	687	419	113	13	1509
Total	1832	2395	709	126	13	5075

serum aminotransferase levels are neither specific nor sensitive enough for diagnosis of NAFLD [2, 24, 25], which was consistent with the present study.

AAR was significantly lower in subjects with NAFLD compared with those without NAFLD. Similarly, lower values for AAR were found in NAFLD subjects compared

Table 5 Distribution of subjects according to FIB-4 index

	FIB-4 index			Total
	<1.3	≥1.3 but <2.67	≥2.67	
Non-NAFLD	2495	1011	60	3566
NAFLD	1117	352	40	1509
Total	3612	1363	100	5075

with those with alcoholic liver disease; thus, AAR can be used to differentiate between these conditions [26]. Another study demonstrated the significance of AAR, even within the spectrum of NAFLD, as a lower AAR was associated with a higher histopathological degree of hepatic steatosis in obese NAFLD subjects [27].

Although standard body weight is determined differently depending on ethnicity, obesity indicates excessive fat accumulation, and there is a relationship between the degree of obesity and the incidence of dyslipidemia, type 2 diabetes mellitus, and hypertension. For example, in Japan, a BMI of 22 is used to indicate the ideal body weight, because the incidence of obesity-related diseases is observed least frequently when the BMI is approximately 22.5 [28]. The incidence of obesity-related diseases is significantly increased in subjects with a BMI of more than 23 in Hong Kong [29]. In the current study, the prevalence of NAFLD showed a linear increase even though each variable was within the normal range. These results suggest that there is no threshold for the incidence of NAFLD and there are differences in the incidence of NAFLD among subjects.

In the present study, we focused on the gender difference for the relationship between the prevalence of NAFLD and metabolic abnormalities. It is well known that NAFLD and NASH exhibit age and sex differences in both prevalence and severity [30]. These age and gender differences are caused by differences in the prevalence of obesity and lifestyle-related diseases [31].

Computed tomography and magnetic resonance imaging are the most reliable procedures for measuring hepatic fat accumulation, but these procedures are not simple enough that they can be used for mass screening. Ultrasonography has many advantages for mass screening. Although ultrasonography is probably the least reliable of these three imaging methods for the quantitative assessment of the degree of hepatic steatosis, ultrasonography is simple and sensitive enough to evaluate hepatic fat accumulation when typical findings of hepatic steatosis are detected. A previous study indicated that the use of ultrasonography for diagnosing NAFLD had a sensitivity of 89% and specificity of 93% for the identification of fatty liver [32].

The prevalence of NASH in the general population is still not clearly documented. A recent study revealed that NASH was confirmed in 12.2% of a largely middle-aged population and 29.9% of patients with ultrasonographic fatty liver [9]. An autopsy study from the late 1980s found that the prevalence of NASH was 2.7% among lean subjects, rising to 18.5% among markedly obese patients [33]. More recently, three studies evaluating donor livers before transplantation found that the prevalence of NASH was 1.1–14% [34–36]. Since it is known that almost 10–20% of subjects with NAFLD have NASH, the prevalence of NASH is estimated to be 13% of the adult Japanese population, which is an extremely large number of potential patients [19]. However, no studies have estimated the prevalence of NASH in the Japanese general population.

In the present study, the prevalence of NAFLD with advanced fibrosis determined as a BAAT ≥3 was predicted as 8.3% in individuals with NAFLD and 2.8% in all subjects. There were 0.4% of subjects with a BAAT ≥3 in the cohort without NAFLD. Further analysis is required to clarify the characteristics of those subjects. The FIB-4 index was developed as a noninvasive panel to stage liver disease in subjects with human immunodeficiency virus and hepatitis C virus co-infection [14]. It has recently been demonstrated that its performance characteristics for the diagnosis of advanced fibrosis in NAFLD are better than those of other similar panels that do not require additional testing, and are comparable with several others that require additional tests [15]. In our study, the estimated prevalence of NASH according to the FIB-4 index was 1.9% in the whole cohort and it was 2.7% in subjects with NAFLD (cutoff ≥2.67). These results, which were predicted using representative scoring indices, suggest that there are potential patients with advanced NASH in the general population and the prevalence is similar to previous studies in Japan [31].

Recently, Sumida et al. [37] suggested a novel scoring system determined by serum ferritin, insulin, and type IV collagen 7S levels (NAFIC score) conducted with Japanese NAFLD patients. Although the scoring system is expected to accurately predict NASH with advanced liver fibrosis, we could not use the NAFIC score because of the lack of parameters.

Some limitations of this study should be noted. First, its cross-sectional design precluded any causal and temporal inferences about the relationships between the presence of NAFLD and various parameters. Second, the diagnosis of NAFLD was made by ultrasonography and exclusion of

other causes of chronic liver disease, but this was not confirmed by liver biopsy, and there were some limitations as mentioned above. Imaging modalities have several limitations in this respect. There might be some possible errors to examine due to the difference of ultrasonography equipment and examiners among each medical facility. The most important limitations of ultrasonography are that (1) it might detect only moderate to severe steatosis, which affects more than one-third of hepatocytes, and it cannot detect mild steatosis, (2) it is difficult to determine an accurate quantitative diagnosis, and (3) there might be differences in measurement deviations in each examiner in a multicenter study, even though a common ultrasonographic definition of NAFLD has been established [38]. Third, there was a lack of some important parameters required to evaluate the background of NAFLD such as waist circumference. We have previously demonstrated a relationship between visceral fat accumulation and development of insulin resistance in patients with NAFLD [39, 40]. Because the relationship between the pathogenesis of NAFLD and visceral fat accumulation is important, further studies are required to clarify the relationship between the prevalence of NAFLD and visceral fat accumulation and the prevalence of metabolic syndrome.

In conclusion, the present study showed that the prevalence of NAFLD is high in the general population in Japan and has increased compared with previous studies, especially in males, even though subjects are not obese. There is a linear relationship between the prevalence of NAFLD and various metabolic parameters, even in nonobese subjects. The prevalence of NASH with advanced fibrosis is estimated to be considerably high in subjects with NAFLD in Japan.

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Conflict of interest None.

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Original Article

Skin toxicities and survival in advanced hepatocellular carcinoma patients treated with sorafenib

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Aim: Sorafenib is the first small molecule with significant clinical activity for advanced hepatocellular carcinoma (HCC). However, intolerable adverse events are sometimes observed. On the other hand, it has been reported that some toxicities of molecular targeted drugs, such as skin toxicities and arterial hypertension, are correlated with good clinical outcomes in other cancers.

Methods: We identified the correlations between adverse events and prognosis for sorafenib therapy in all patients with HCC treated at the institutions of the Saga Liver Cancer Study Group. The toxicities were assessed using the Common Terminology Criteria for Adverse Events version 4.0.

Results: Ninety-four patients received sorafenib until August 2010. The overall incidence of treatment-related adverse events was 98% of patients. Skin toxicities, including palmar-plantar erythrodysesthesia syndrome, rash, pruritus and alopecia, were the most common adverse events and were observed in 58 patients (62%). Hypertension was observed in 23 patients (24%). The median survival time was 12.5 months among the total patients. The patients with skin toxicities showed significantly longer survival than the patients without these toxicities (hazard ratio, 0.449; 95% confidence interval, 0.256–0.786; $P = 0.005$). Hypertension had no correlation with survival. Skin toxicities were also significant prognostic factors in a multivariate analysis (hazard ratio, 0.522; 95% confidence interval, 0.274–0.997; $P = 0.049$), along with Child–Pugh class and α-fetoprotein level. The median development time for skin toxicities was 21 days.

Conclusion: Skin toxicities occur commonly at the early phase in patients treated with sorafenib, and could be a promising surrogate marker for the treatment outcome.

Key words: adverse event, chemotherapy, liver carcinoma

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common cancers worldwide.[1,2] It occurs frequently in the Asia–Pacific and Africa regions, and ranges 50–150/100 000 people per year. The prognosis of HCC depends on the stage of HCC at the time of diagnosis. One of the most widely used staging systems is the Barcelona Clinic Liver Cancer (BCLC) classification,[3] which comprises four categories: stage A, early HCC; stage B, intermediate HCC; stage C, advanced HCC; and stage D, end-stage HCC. Although patients with early HCC who can receive radical therapy have a good prognosis, patients with advanced HCC have a poor clinical outcome.

Sorafenib is the first targeted agent with significant clinical activity for advanced HCC. It is a small molecule that inhibits the activities of the serine-threonine kinases Raf-1 (c-Raf) and B-Raf; the receptor tyrosine kinases vascular endothelial growth factor receptor (VEGFR)-1, -2 and -3; and platelet-derived growth factor receptor (PDGFR)-α and -β.[4] Previous multicenter,

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double-blind, randomized phase III studies, the SHARP study[5] and the Asia–Pacific study,[6] showed statistically significant survival benefits compared with placebo in patients with advanced HCC, with hazard ratios of approximately 0.7. In these trials, sorafenib was characterized by a good tolerability profile, although intolerable adverse events were sometimes observed.[7] On the other hand, it has been reported that some toxicities of targeted agents, such as skin toxicities[8–15] and arterial hypertension,[16–19] are correlated with good clinical outcomes in other cancers. The aim of this study was to identify the correlations between these adverse events and prognosis for sorafenib treatment in patients with HCC.

METHODS

Patients and treatment

A RETROSPECTIVE ANALYSIS for all patients with HCC treated with sorafenib in Saga Prefecture was performed using the unified database system of the Saga Liver Cancer Study Group, which is composed of tertiary-care hospitals with specialists in liver cancer treatment in Saga Prefecture. All patients had histologically or radiologically confirmed HCC that was diagnosed as advanced, ineligible for resection or locoregional treatment, or refractory to chemoembolization. The inclusion criteria were Eastern Cooperative Oncology Group (ECOG) performance status scores of 0–2, Child–Pugh scores of up to 8, and adequate hematologic and liver functions. Adequate hematologic functions were defined as a hemoglobin level of 8.5 g/dL or more, neutrophil count of more than 1500/μL and platelet count of more than 75 000/μL. Adequate liver functions were defined as alanine aminotransferase and aspartate aminotransferase levels of less than fivefold the normal upper limit and total bilirubin level of less than 2.0 mg/dL. Patients requiring hemodialysis were excluded. Patients were also considered ineligible if they received concomitant systemic therapy, including any targeted agents. The institutional review board or ethics committee of each institution approved this study protocol. All patients provided written informed consent before the treatment.

The patients received 400 mg of sorafenib twice daily. Initial dose reductions with consideration of each patient's condition were allowed. The treatment was continued until disease progression or intolerable drug-related toxicities occurred. Dose reductions (first to 400 mg once daily, and then to 400 mg every 2 days) and interruptions were permitted for drug-related toxicities.

Assessment

Toxicities were assessed using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The patients were divided into groups according to the presence or absence of skin toxicities and hypertension related to sorafenib. Skin toxicities were considered to be palmar-plantar erythrodysesthesia syndrome, rash, pruritus and alopecia of at least grade 1 according to the CTCAE version 4.0. Patients with sorafenib-related hypertension were also chosen as at least grade 1. The treatment effects were then evaluated and compared between the groups. Radiological evaluations were carried out every 4–8 weeks using enhanced computed tomography or magnetic resonance imaging according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1.[20]

Statistical analysis

The proportions and antitumor effects between the two groups were compared using the Mann–Whitney *U*-test for continuous data and Cochran–Mantel–Haenszel χ^2-test for categorical data. The time to progression was calculated from the date of administration of sorafenib to the date of radiological progression or was censored at either the last follow up or at the time of death without evidence of radiological progression. The overall survival time was calculated from the date of administration of sorafenib to the date of death from any cause or was censored at the last follow up. The time to progression and survival time were estimated using the Kaplan–Meier method, and the survival curves were compared using the log-rank test. The treatment effects were adjusted using the Cox proportional hazards model. Differences with values of $P < 0.05$ were considered statistically significant. Data analyses were performed using R version 2.12.2 (The R Foundation for Statistical Computing).

RESULTS

Patient population and outcomes

SORAFENIB MONOTHERAPY WAS initiated in 94 patients from July 2008 to August 2011, and their characteristics are shown in Table 1. Eighty-seven patients (93%) had a history of HCC treatment before sorafenib therapy. There were seven treatment-naïve patients with a BCLC stage of C. The Child–Pugh class of