by hepatocytes, thereby causing diabetes (23). Steatosis occurs more frequently in patients with chronic HCV infection than in those with chronic HBV infection; this may explain the increased risk of DM among HCV patients (24). Although we proposed possible explanations for the correlation between HCV infection and the prevalence rate of type 2 DM in patients in this study, it is also possible that the mechanism is multifactorial. A previous study identified chronic hepatitis B as having no relationship to DM, and on the basis of the results of this study, we arrive at the same conclusion (25,26).

Several studies have indicated that the progression from chronic hepatitis to cirrhosis and HCC is accelerated by dual HCV infection (11,27). The strong effect of DM on HCC in the absence of hepatitis infection suggests that, in addition to the hepatitis C causal pathway, HCC is mediated through the reduction of IGF-1 factors or IGF binding protein-3, caused by hyperinsulinemia. This in turn stimulates the proliferation of cancer cells, as demonstrated by Lagiou *et al* (28). In the present study, the prevalence rate of DM in patients with HCC-C was significantly higher in patients older than 66 years of age. Our findings demonstrate that the effects of the interaction between DM and HCV further the incidence of HCC.

In conclusion, the prevalence of HCC-nonBC and HCC-C was significantly higher than that of HCC-B, while the prevalence of HCC-nonBC was significantly higher than that of HCC-C. In patients over 66 years of age, the prevalence of type 2 DM in HCC-B and HCC-nonBC cases was increased, whereas the prevalence of type 2 DM in HCC-C cases was significantly decreased. Our findings indicate that the interaction between type 2 DM and HCV increases the prevalence of HCC.

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The incidence of hepatocellular carcinoma associated with hepatitis C infection decreased in Kyushu area

Naota Taura[1ABCDEFG], Nobuyoshi Fukushima[2ABCDEFG], Hiroshi Yatsuhashi[1ABCDEFG], Yuko Takami[3ABCDEFG], Masataka Seike[4ABCDEFG], Hiroshi Watanabe[5ABCDEFG], Toshihiko Mizuta[6ABCDEFG], Yutaka Sasaki[7ABCDEFG], Kenji Nagata[8ABCDEFG], Akinari Tabara[9ABCDEFG], Yasuji Komorizono[10ABCDEFG], Akinobu Taketomi[11ABCDEFG], Shuichi Matsumoto[12ABCDEFG], Tsutomu Tamai[13ABCDEFG], Toyokichi Muro[14ABCDEFG], Kazuhiko Nakao[15ABCDEFG], Kunitaka Fukuizumi[16ABCDEFG], Tatsuji Maeshiro[17ABCDEFG], Osami Inoue[18ABCDEFG], Michio Sata[2ABCDEFG]

[1] Clinical Research Center, National Nagasaki Medical Center, Omura City, Nagasaki, Japan
[2] Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Kurume City, Fukuoka, Japan
[3] Department of Surgery, National Hospital Organization Kyushu Medical Center, Chuo-ku, Fukuoka City, Fukuoka, Japan
[4] 1st Department of Internal Medicine, Oita University Faculty of Medicine, Hasama-machi, Yufu City, Oita, Japan
[5] Department of Hepatology, Fukuoka Red Cross Hospital, Minami-ku, Fukuoka City, Fukuoka, Japan
[6] Department of Internal Medicine, Saga University Faculty of Medicine, Saga City, Saga, Japan
[7] Department of Gastroenterology and Hepatology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto City, Kumamoto, Japan
[8] Department of Internal Medicine II, Miyazaki Medical College, Kiyotake-cho, Miyazaki-gun, Miyazaki, Japan
[9] 3rd Department of Internal Medicine, University of Occupational and Environmental Health, Kitakyusyu City, Fukuoka, Japan
[10] Department of Hepatology, Nanpuh Hospital, Kagoshima City, Kagoshima, Japan
[11] Department of Surgery and Science, Graduate school of medical sciences, Kyushu University, Fukuoka City, Fukuoka, Japan
[12] Department of Internal Medicine, Fukuoka Tokusyukai Medical Center, Kasuga City, Fukuoka, Japan
[13] Digestive Disease and Life-Style Related Disease, Health Research Human and Environmental Sciences, Kagoshima University, Kagoshima City, Kagoshima, Japan
[14] Department of Gastroenterology, National Hospital Organization Oita Medical Center, Oita City, Oita, Japan
[15] Department of Gastroenterology and Hepatology, Nagasaki University School of Medicine, Nagasaki City, Nagasaki, Japan
[16] Department of Gastroenterology, National Hospital Organization, Kyushu Medical Center, Fukuoka City, Fukuoka, Japan
[17] 1st Department of Internal Medicine, Faculty of Medicine, University of the Ryukyus, Nakagami-gun, Okinawa, Japan
[18] Department of Gastroenterology, Nagasaki Rousai Hospital, Sasebo City, Nagasaki, Japan

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Summary

Background: The incidence of hepatocellular carcinoma (HCC) in Japan has still been increasing. The aim of the present study was to analyze the epidemiological trend of HCC in the western area of Japan, Kyushu.

Material/Methods: A total of 10,010 patients with HCC diagnosed between 1996 and 2008 in the Liver Cancer study group of Kyushu (LCSK), were recruited for this study. Cohorts of patients with HCC were categorized into five year intervals. The etiology of HCC was categorized to four groups as follows; B: HBsAg positive, HCV-RNA negative, C: HCV-RNA positive, HBsAg negative, B+C: both of HBsAg and HCV-RNA positive, non-BC: both of HBsAg and HCV-RNA negative.

Results: B was 14.8% (1,485 of 10,010), whereas 68.1% (6,819 of 10,010) had C, and 1.4% (140 of 10,010) had HCC associated with both viruses. The remaining 1,566 patients (15.6%) did not associate with both viruses. Cohorts of patients with HCC were divided into six-year intervals (1996–2001 and 2002–2007). The ratio of C cases decreased from 73.1% in 1996–2001 to 64.9% in 2002-2007. On the other hand, B and -nonBC cases increased significantly from 13.9% and 11.3% in 1996-2001 to 16.2% and 17.6% in 2002-2007, respectively.

Conclusions: The incidence of hepatocellular carcinoma associated with hepatitis C infection decreased after 2001 in Kyushu area. This change was due to the increase in the number and proportion of the HCC not only nonBC patients but also B patients.

key words: hepatitis virus • hepatocellular carcinoma • Japan

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Author's address: Hiroshi Yatsuhashi, Clinical Research Center, National Nagasaki Medical Center, 2-1001-1 Kubara, Omura City, Nagasaki, Japan, e-mail: yatsuhashi@nmc.hosp.go.jp

Background

The three leading causes of death in Japan are malignancy neoplasms, cardiovascular diseases, and cerebrovascular diseases. Since 1981, malignant neoplasms have been the leading cause of death in Japan. For the last 30 years, liver cancer has been the third leading cause of death from malignant neoplasms in men. In women, liver cancer has ranked fifth during the past decade [1]. Hepatocellular carcinoma (HCC) accounts for 85% to 90% of primary liver cancers[2] and the age-adjusted HCC mortality rate has increased in recent decades in Japan [3]. Similarly, a trend of increasing rates of HCC has been reported from several developed countries in North America, Europe and Asia [4,5]. HCC often develops in patients with liver cirrhosis caused by hepatitis B virus (HBV), hepatitis C virus (HCV), excessive alcohol consumption, or nonalcoholic fatty liver disease. Of the hepatitis viruses which cause HCC, HCV is predominant in Japan [6–9].

Although the age-adjusted incidence of HCC has increased in Japan, sequential changes in etiology of HCC patients between 2001 and 2008 are not fully understood [10]. To clarify factors affecting epidemiological changes in Japanese HCC patients, especially the recent trend of HCC, we analyzed the epidemiological trend of HCC in the western area of Japan, Kyushu area.

Material and Methods

Patients

A total of 10,010 patients with HCC diagnosed between 1996 and 2008 in the Liver Cancer study group of Kyushu (LCSK), were recruited for this study. The diagnosis of HCC was based on AFP levels and imaging techniques including ultrasonography (USG), computerized tomography (CT), magnetic resonance imaging (MRI), hepatic angiography (HAG), and/or tumor biopsy. The diagnostic criteria for HCC were either a confirmative tumor biopsy or elevated AFP (>20 ng/mL) and neovascularization in HAG and/or CT.

Etiology of HCC

A diagnosis of chronic HCV infection was based on the presence of HCV-RNA detected by polymerase chain reaction (PCR), whereas diagnosis of chronic HBV infection was based on the presence of hepatitis B surface antigen (HBsAg). The etiology of HCC was categorized to four groups as follows; **B:** HBsAg positive, HCV-RNA negative, **C:** HCV-RNA positive, HBsAg negative, **B+C:** both of HBsAg and HCV-RNA positive, **nonBC:** both of HBsAg and HCV-RNA negative.

Statistical analysis

The data were analyzed by the Mann-Whitney test for the continuous ordinal data, the x2 test with Yates' correction and the Fisher exact test for the association between two qualitative variables. The standard deviation was calculated based on the binomial model for the response proportion. P<0.05 was considered statistically significant.

Results

Clinical features of the studied patients

A total of 10,010 patients with HCC were diagnosed at our study group from 1996 to 2008. Table 1 show that the proportion of patients diagnosed with **B** was 14.8% (1,485 of 10,010), whereas 68.1% (6,819 of 10,010) had **C**, and an additional 1.4% (140 of 10,010) had HCC associated with both viruses. The remaining 1,566 patients (15.6%) did not associate with both viruses. In analysis of patients in HCC by category, the median age of patients at diagnosis of **B** was 57 years old significant younger than other types HCC(**C: 69, nonBC: 70,.B+C 65 years old).**

As shown in Figures 1 and 2, the number and ratio of **B** cases remained unchanged from1996 to 2001 and thereafter increased and plateaued, whereas **C** rapidly increased from 1996 to 2000 and thereafter decreased and plateaued. In addition, the number and ratio of the **nonBC** cases has increased continued gradually and continued in this study period.

Change of etiology in patients with HCC during the period 1996–2007 with 6-years intervals

Cohorts of patients with HCC were divided into six-year intervals (1996–2001 and 2002–2007). Table 2 show that the incident rate of **C** decreased significantly from 73.1% in 1996-2001 to 64.9% in 2002-2007 (1996–2001 vs. 2002–2007, p=<0.001). On the other hand, the incident rate of **B** and **-nonBC** increased significantly from 13.9% and 11.3% in 1996-2001 to 16.2% and 17.6% in 2002-2007, respectively. Not only the incident rate but also number of **B** and **-nonBC** became larger in same 6 years periods.

Table 3 shows that male/female ratio of **C** and **-nonBC** decreased significantly from 2.2 and 4.0 in 1996-2001 to 1.8 and 2.7 in 2002-2007, respectively (p<0.001). The ratio became clearly smaller, indicates an increase in female patients with **C** and **nonBC.** On the other hand, the male/female ratio of **B** patients did not significantly change during the period. The median age at diagnosis of **B, C,** and **nonBC** in six-year intervals were significant increase from 56 to 58, from 67 to 71 and from 68 to 71 years of age during the period.

Discussion

Our study was the twenty-three major liver center-based study designed to examine the sequential change in the background of HCC patients during the past 13 years, 1996–2008. More than 80% of our patients had chronic HBV or HCV infections. During this observation period, the number and proportion of HCC-C reached a peak in 2000 and thereafter decreased and became stabilized. Previous studies from Japan reported that the proportion of the HCC patients with HCV infection had been increased and reached a plateau in the period of 1981–2001 [1,3,10–12]. However, in our study, the number and proportion of the HCC patients with HCV infection cases decreased in 2001–2008. The reason may be explained as follows; interferon therapy for chronic hepatitis C may have been associated with a decreased incidence of HCC [13–17]. Oral supplementation with a oral branched-chain amino acids has been useful in the prevention HCC [18]. Finally, the chronically HCV-infected

Table 1. The characteristic of HCC patients during the period of 1996–2008.

Age (y.o.)	B		C		nonB		B+C		Total
	Male	Female	Male	Female	Male	Female	Male	Female	
0–	1	0	0	1	0	0	0	0	2
10–	4	1	0	0	0	2	0	0	7
20–	6	2	1	0	1	1	0	0	11
30–	31	5	4	0	11	3	2	0	56
40–	204	22	130	12	32	15	12	0	427
50–	507	66	728	145	167	32	31	6	1,682
60–	287	118	1836	741	411	102	35	13	3,543
70–	140	64	1775	947	483	133	22	14	3,578
80–	9	18	271	214	97	65	1	4	679
90–	0	0	9	5	9	2	0	0	58
Total	**1,189**	**296**	**4,754**	**2,065**	**1,211**	**355**	**103**	**37**	**10,010**
	1,485 (4.8%)		6,819 (68.1%)		1,566 (15.6%)		140 (1.4%)		
Median	57	63	67	70	68	70	61	68	67
	57		69		70		65		
Mean	56	64	68	71	69	71	62	68	67
	58		68		68		63		
Range	1–87	14–89	27–94	0–93	28–96	17–90	36–82	55–82	0–96
	1–89		0–94		17–96		36–82		

Age: B vs. C $p \leq 0.001$; B vs. B+C $p \leq 0.001$; B vs. nonBC $p \leq 0.001$; C vs. BC $p \leq 0.001$; C vs. nonBC $p=0.043$; BC vs. nonB+C $p \leq 0.001$. IQR – interquartile range; SD – standard deviation.

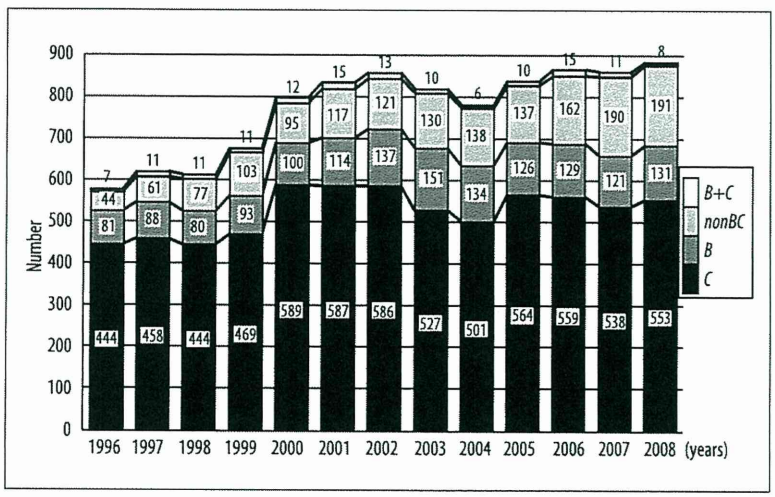

Figure 1. Sequential changes in the number of HCC patients categorized by etiology during the period 1996–2008.

population is aging in Japan. Yoshizawa et al. reported that age-specific prevalence for the presence of HCVAb among ~300,000 voluntary blood donors from Hiroshima in 1999 clearly increased with the age, reaching the highest proportion of 7% in individuals who were more than 70 years old [10,19]. In this study, the median age of the HCC patients with HCV infection steadily increased from 67 to 71 years of age during the studied period. In a word, HCV infected people become older with years in Japan and they were regarded as a high risk for HCC.

The prevalence rate of HBV in Kyushu area has been reported to be higher than other area in Japan [1]. In Kyushu area, 95% of patients with chronic HBV infection had HBV genotype C except for Okinawa [20]. HBV genotype C is thought to be associated with higher incidence of HCC

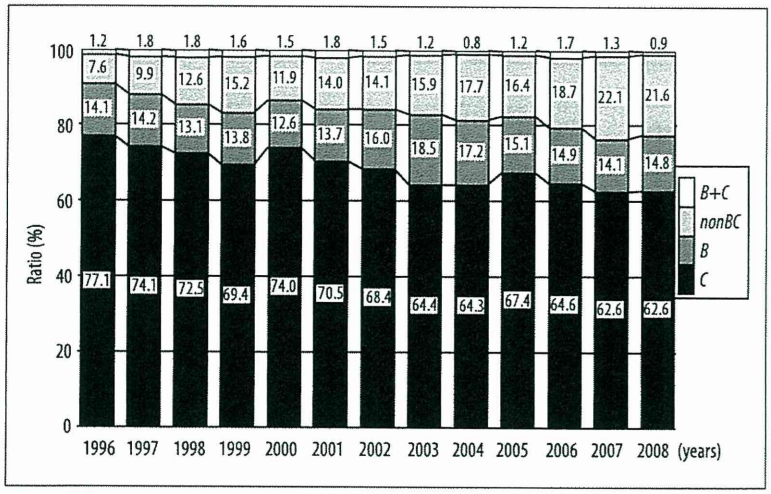

Figure 2. Sequential changes in the ratio of HCC patients categorized by etiology during the period 1996–2008.

Table 2. Change of etiology in patients with HCC during the period 1996–2007 with 6-years intervals.

Period		1996–2001	2002–2007	P value
Number		3,023	4,173	
Sex	Male	2,162	2,849	
	Female	861	1,324	
Ratio (male/female)		2.5	2.2	0.003
Age (y.o.) (IQR)		66 (14)	69 (12)	<0.001
Hepatitis virus (%)	B	13.9	16.2	
	C	73.1	64.9	
	B+C	1.7	1.3	
	nonBC	11.3	17.6	0.001

QR – interquartile range.

Table 3. The median age and male/female ratio of HCC patients during the period of 1996–2007.

Period		1996–2001	2002–2007	P value
B				
Age (y.o.) (IQR)		56 (14)	58 (15)	0.001
Sex	Male	331	519	
	Female	88	157	
Ratio (male/female)		3.8	3.3	0.391
C				
Age (y.o.) (IQR)		67 (9)	71 (11)	<0.001
Sex	Male	1,524	1,753	
	Female	687	955	
Ratio (male/female)		2.2	1.8	0.002
nonBC				
Age (y.o.) (IQR)		68 (12)	71 (13)	<0.001
Sex	Male	273	534	
	Female	69	201	
Ratio (male/female)		4.0	2.7	0.012

QR – interquartile range.

compared with other HBV genotypes [21]. In the present study, the incident rate of HCC patients with HBV infection became larger in this study period. To explain this change, we must consider from two viewpoints. The one is that the number of patients with HCC caused by HCV infection decreased, the other is that the proportion of chronic HBV infected patients who have reached the age of developing HCC is relatively high as described below.

Nationwide health survey for HBsAg in the over 40 years of age population had been done between 2002 and 2006 in Japan. This survey reports indicated that the average HBsAg prevalence was 1.2% in the total Japanese population patients with chronic HBV infection [10] and the age-specific prevalence of HBsAg was higher in the group aged between 50 (1.4%) and 55 years (1.5%). In the HCC patients with HBV genotype C, the mean age was 55 years in Japan [20]. This overlap between age-specific prevalence and hepatocellular carcinogenic age would be associated with the increase of HCC patients with HBV infection. Nucleoside analogue reverse transcriptase inhibitor (NARTI) therapy effectively reduces the incidence of HCC in chronic hepatitis B patients [22,23]. However, Interferon therapy for chronic hepatitis C started from 1992, whereas NARTI therapy for HBV started from 2000 in Japan [24,25]. Hence, HBV associated HCC will probably decrease in Japan during the next 10 to 20 years.

The survey of HCC patients associated with nonBC infection in Japan was conducted by Inuyama Hepatitis Research Group from 1995 to 2003. The ratio of HCC patients with nonBC accounted 9.3% [1]. In the present study, the ratio of HCC patients with nonBC was 14.1%. Furthermore, the number and the proportion of HCC patients with nonBC have been gradually increasing in the periods. The current two studies account for the increase in number and proportion of HCC patients with nonBC. First, Lai et al. reported

that type 2 diabetes increases the risk of developing HCC in those who are HCV negative or have a high level of total cholesterol [26]. Second, Nakano et al. reported that epidemiological studies on diabetes mellitus revealed that the number of patients with diabetes mellitus is gradually increasing in Japan along with development of car society and westernization of food intake. Since prevalence of diabetes mellitus increases with aging, proportion of individuals with diabetes mellitus aged over 60 has exceeded two-third of estimated total number of patients (7.40 million in 2002) in Japan where aging of society is rapidly progressing [27]. In a word, the number of type 2 diabetes people is increasing in Japan and they were regarded as a high risk for HCC. Then, the number and the proportion of HCC patients with nonBC have been increased recent twelve years in Japan.

It is known that 2 to 4 decades of chronic HCV infection are required to develop cirrhosis and subsequent HCC [28–31]. The number of HCC cases has increased in Japan, because individuals infected with HCV during the past have grown old and have reached the cancer-bearing age. The prevalence of HCV infection in young Japanese individuals is low and the incidence of HCVAb is very low because of preventative actions against HCV infection such as the screening of blood products for HCV and the use of sterile medical equipment [32]. Additionally, we showed that the number and proportion of patients with HCC-C cases decreased, whereas the number and ratio of HCC-nonBC steadily increased during the studied period. These findings may be expected that the incidence of HCC patients with nonBC in Japan may continue to increase even after the consequence of the HCV epidemic level off, a country that is far advanced with regard to HCC patients with HCV infection, in the near future.

Conclusions

In summary, HCC patients had increased from 1996 to 2000 and this increase was originated from HCC patients with HCV infection. The number and proportion of HCC patients with HCV infection reached a peak in 2000 and thereafter decreased and became stabilized. The incidence of hepatocellular carcinoma associated with hepatitis C infection decreased after 2001 in Kyushu area. This change was due to the increase in the number and proportion of the HCC not only nonBC patients but also B patients.

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Original Article

Data mining reveals complex interactions of risk factors and clinical feature profiling associated with the staging of non-hepatitis B virus/non-hepatitis C virus-related hepatocellular carcinoma

Takumi Kawaguchi,[1] Tatsuyuki Kakuma,[2] Hiroshi Yatsuhashi,[3] Hiroshi Watanabe,[4] Hideki Saitsu,[5] Kazuhiko Nakao,[6] Akinobu Taketomi,[7] Satoshi Ohta,[8] Akinari Tabaru,[9] Kenji Takenaka,[10] Toshihiko Mizuta,[11] Kenji Nagata,[12] Yasuji Komorizono,[13] Kunitaka Fukuizumi,[14] Masataka Seike,[15] Shuichi Matsumoto,[16] Tatsuji Maeshiro,[17] Hirohito Tsubouchi,[18] Toyokichi Muro,[19] Osami Inoue,[20] Motoo Akahoshi[21] and Michio Sata:[1] The Liver Cancer Study Group of Kyushu

[1]*Department of Digestive Disease Information and Research and Department of Medicine, Kurume University School of Medicine,* [2]*The Biostatistics Center, Medical School, Kurume University, Kurume,* [3]*Department of Therapeutic Research, Clinical Research Center, National Hospital Organization Nagasaki Medical Center, Omura,* [4]*Hepatology Division, Fukuoka Red Cross Hospital,* [5]*Department of Surgery, Center for Liver Diseases, National Hospital Organization Kyushu Medical Center, Fukuoka,* [6]*Department of Gastroenterology and Hepatology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki,* [7]*Department of Surgery and Science, Kyushu University,* [8]*Division of Gastroenterology, National Kyushu Cancer Center, Fukuoka,* [9]*Third Department of Internal Medicine, University of Occupational and Environmental Health, Japan, School of Medicine, Kitakyushu,* [10]*Fukuoka City Hospital, Fukuoka,* [11]*Department of Internal Medicine, Saga University, Saga,* [12]*Gastroenterology and Hematology, Internal Medicine, Faculty of Medicine, University of Miyazaki, Miyazaki,* [13]*Hepatology, Nanpuh Hospital, Kagoshima,* [14]*Department of Gastroenterology, National Hospital Organization Kyushu Medical Center, Fukuoka,* [15]*Department of Internal Medicine I, Faculty of Medicine, Oita University, Yufu,* [16]*Department of Internal Medicine, Fukuoka Tokushukai Medical Center, Fukuoka,* [17]*Department of Infections, Respiratory, and Digestive Medicine Control and Prevention of Infectious Disease Faculty of Medicine, University of the Ryukyus, Okinawa,* [18]*Department of Digestive and Life-style Related Disease, Kagoshima University Graduate School of Medical and Dental Scienses, Kagoshima,* [19]*Department of Gastroenterology, National Hospital Organization Oita Medical Center, Oita,* [20]*Digestive Organ Center, Nagasaki Labour Welfare Hospital, Sasebo, and* [21]*Department of Internal Medicine, Nishinihon Hospital, Kumamoto, Japan*

Aim: Non-hepatitis B virus/non-hepatitis C virus-related hepatocellular carcinoma (NBNC-HCC) is often detected at an advanced stage, and the pathology associated with the staging of NBNC-HCC remains unclear. Data mining is a set of statistical techniques which uncovers interactions and meaningful patterns of factors from a large data collection. The aims of this study were to reveal complex interactions of the risk factors and clinical feature profiling associated with the staging of NBNC-HCC using data mining techniques.

Methods: A database was created from 663 patients with NBNC-HCC at 20 institutions. The Milan criteria were used as staging of HCC. Complex associations of variables and clinical feature profiling with the Milan criteria were analyzed by graphical modeling and decision tree algorithm methods, respectively.

Results: Graphical modeling identified six factors independently associated with the Milan criteria: diagnostic year of HCC; diagnosis of liver cirrhosis; serum aspartate aminotransferase (AST); alanine aminotransferase (ALT); α-fetoprotein (AFP); and des-γ-carboxy prothrombin (DCP) levels. The decision trees were created with five variables to classify six groups of patients. Sixty-nine percent of the patients were

Correspondence: Dr Takumi Kawaguchi, Department of Digestive Disease Information and Research, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan. Email: takumi@med.kurume-u.ac.jp
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within the Milan criteria, when patients showed an AFP level of 200 ng/mL or less, diagnosis of liver cirrhosis and an AST level of less than 93 IU/mL. On the other hand, 18% of the patients were within the Milan criteria, when patients showed an AFP level of more than 200 ng/mL and ALT level of 20 IU/mL or more.

Conclusion: Data mining disclosed complex interactions of the risk factors and clinical feature profiling associated with the staging of NBNC-HCC.

Key words: data mining, disease progression, hepatoma, non-viral hepatitis, tumor marker

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is the fifth most common cancer and the third most common cause of cancer-related deaths worldwide.[1–3] Chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) is a risk factor for HCC. Recent developments in the management of patients with viral hepatitis have resulted in early detection of HCC and improvement of prognosis.[4–8]

The number of patients with non-HBV/non-HCV-related HCC (NBNC-HCC) has been increasing, and NBNC-HCC now accounts for 12–16% of all the HCC cases in Japan.[8,9] A variety of factors are involved in the development and progression of this cancer including age, sex, alcoholic liver disease and diabetes mellitus.[10–12] Therefore, neither early detection nor improved prognosis has been achieved in NBNC-HCC.[6] Radical treatment is applicable to patients with NBNC-HCC who meet the Milan criteria;[13] however, this cancer is often detected at an advanced stage. For earlier detection, it is important to understand the complex interactions of the risk factors and clinical feature profiling associated with the Milan criteria, a staging system for NBNC-HCC.

Data mining, a set of statistical techniques, uncovers meaningful patterns and interactions of variables from a large data collection even when there is no a priori hypothesis imposed.[13] Graphical modeling is an exploratory multivariate analysis of data mining that reveals complex associations between variables.[14] This analysis assumes that the response variable is influenced by multiple factors.[15] Therefore, different from results of univariate analysis, an association between a risk factor and an outcome variable may disappear or appear because of the effects of another set of variables known as "confounding factors".[16,17] Furthermore, its findings are visualized as a graph, which provides an idea of how variables interact and denotes the conditional independence structure between random variables.[15] Therefore, graphical modeling is now identified as a new approach to model clinical data.[18]

Decision tree making is another exploratory technique of data mining that represents a series of rules for classification by identifying priorities.[19–21] It is an explicit, quantitative and systematic approach to decision-making under conditions of uncertainty and allows clinicians to choose an option that maximizes the net benefit to the patient.[22] Recently, decision trees were used to reveal the clinical feature profiling for staging of pancreatic cancer[23] and ovarian cancer.[24] However, decision trees have never been applied to identify the clinical feature profiling associated with the staging of NBNC-HCC.

The aims of this study were to reveal complex interactions of the risk factors and clinical feature profiling associated with the staging of NBNC-HCC using data mining techniques.

METHODS

Patient database

BETWEEN 1995 AND 2006, a total of 10 133 patients were diagnosed with HCC at 23 institutions located in Kyushu, a high morbidity area of HCC in Japan. Among them, 1363 patients were diagnosed with NBNC-HCC according to the negative results of both serum hepatitis B surface antigen and serum anti-HCV antibody or HCV RNA.

In order to examine the clinical variables associated with the staging of NBNC-HCC, a database of 663 patients with NBNC-HCC at 20 institutions was created on the basis of the following variables: diagnostic year of HCC; age; sex; family history of liver disease; past history of blood transfusion; alcohol intake; diagnosis of liver cirrhosis; diagnosis of liver disease; diagnosis of diabetes mellitus; serum aspartate aminotransferase (AST) level; serum alanine aminotransferase (ALT) level; serum α-fetoprotein (AFP) level; serum des-γ-carboxy prothrombin (DCP) level; size of HCC; and number of HCC.

For practical use, alcohol intake, serum AFP level and serum DCP level were categorized as follows. Alcohol intake: none; 60 g/day or less; 60–100 g/day; or more than 100 g/day. AFP level: 20 ng/mL or less; 20–200 ng/mL; or more than 200 ng/mL. DCP level: 40 mAU/mL or less; 40–100 mAU/mL; or more than 100 mAU/mL.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected by the approval of the Ethics Committee of the Kurume University School of Medicine.

Diagnosis and staging of HCC

The diagnosis of HCC was based on the clinical practice manual proposed by the Japan Society of Hepatology,[25] by using serum AFP and DCP levels and imaging techniques including ultrasonography, computerized tomography, magnetic resonance imaging, hepatic angiography and/or tumor biopsy. The Milan criteria (single nodule ≤5 cm or three nodules <3 cm) were used for the staging of HCC.[26]

Data mining

An association between the Milan criteria and each risk factor was examined by Student's *t*-test and χ^2-test. Because of the insufficient scientific evidence for testing specific clinical hypotheses, graphical modeling and decision trees were employed to explore complex associations between the Milan criteria and a set of risk factors.

MIM software (http://www.hypergraph.dk/) was used for graphical modeling. R package rpart (recursive partitioning and regression trees by Terry Therneau and Beth Atkinson; http://www.mayo.edu/biostatistics) was used to construct a decision tree algorithm. In order to evaluate the prediction error, the original data ($n = 663$) were randomly divided into a training dataset ($n = 442$) and a test dataset ($n = 221$). Ten-hold cross-validation was conducted to construct the initial tree on the basis of the training dataset; then, the optimal-size tree was constructed by examining a set of cost–complexity parameters. The overall prediction error rate as well as the sensitivity and specificity were calculated by applying the results of the decision tree algorithm to the test dataset.

RESULTS

Characteristics of patients with NBNC-HCC

THE PATIENTS' CHARACTERISTICS are summarized in Table 1. Family history of liver disease and history of blood transfusion were not noted in more than 80% of the patients. Approximately 40% of the patients did not have any etiology of chronic liver disease.

Univariate analysis of variables associated with the Milan criteria

Univariate analysis showed that diagnosis of liver cirrhosis, serum AST level, serum ALT level, serum AFP level and serum DCP level were significantly associated with the Milan criteria (Table 2).

Table 1 Characteristics of all patients

Variable	
n	663
Diagnostic year of HCC (years)	2002 ± 3
Age (years)	68.1 ± 9.9
Male/female	480/183
Family history of liver disease (yes/no/unclear)	79/547/37
History of blood transfusion (no/before 1989/after 1989/unclear)	584/29/22/28
Daily alcohol intake (none/<60 g/60–100 g/>100 g)	254/183/141/85
Etiology of chronic liver disease (none/alcohol/others)	296/188/179
Diagnosis of liver cirrhosis (yes/no)	260/403
Diagnosis of diabetes mellitus (no/yes without medication/yes with medication)	396/109/158
Serum AST level (U/L)	53.3 ± 51.3
Serum ALT level (U/L)	51.8 ± 49.9
Serum AFP level (ng/mL)	9397 ± 71066
Serum DCP level (mAU/mL)	8003 ± 37377
Size of HCC (cm)	5.0 ± 3.4
Number of HCC	2.8 ± 2.9

Data are expressed as the mean ± standard deviation or the number of patients.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma.

Graphical modeling

Complex interactions of the risk factors associated with the Milan criteria were visualized graphically (Fig. 1). Graphical modeling identified six independent factors directly associated with the Milan criteria: diagnostic year of HCC; diagnosis of liver cirrhosis; serum AST level; serum ALT level; serum AFP level; and serum DCP level (Fig. 1). Although alcohol intake, diagnosis of liver disease and diagnosis of diabetes mellitus were not directly associated with the Milan criteria, they were associated with the Milan criteria through diagnosis of liver cirrhosis (Fig. 1).

Decision tree algorithm

With the training dataset ($n = 442$), a decision tree algorithm was created by using five variables to classify six groups of patients (Fig. 2). A serum AFP level of 200 ng/mL or less was the cut-off value for the initial

Table 2 Univariate analysis of the variables associated with the Milan criteria

Variable	Statistical method	Test statistics	Degree of freedom (df)	*P*
Diagnostic year of HCC (years)	χ^2	13.4013	11	0.2679
Age (years)	Pooled	−1.07	661	0.2843
Sex	χ^2	0.2975	1	0.5854
Family history of liver disease	χ^2	1.7412	1	0.187
History of blood transfusion	χ^2	4.9527	2	0.084
Daily alcohol intake	χ^2	2.4158	3	0.4907
Liver cirrhosis	χ^2	28.9521	1	<0.0001
Diabetes mellitus	χ^2	0.926	2	0.6294
AST level (U/L)	Satterthwaite	3.06	387.51	0.0023
ALT level (U/L)	Satterthwaite	4.79	546.95	<0.0001
AFP level (ng/mL)	χ^2	63.1357	2	<0.0001
DCP level (mAU/mL)	χ^2	47.7161	2	<0.0001

Associations between the variables and the Milan criteria were analyzed by the indicated statistical methods. $P < 0.05$ was considered significant.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma.

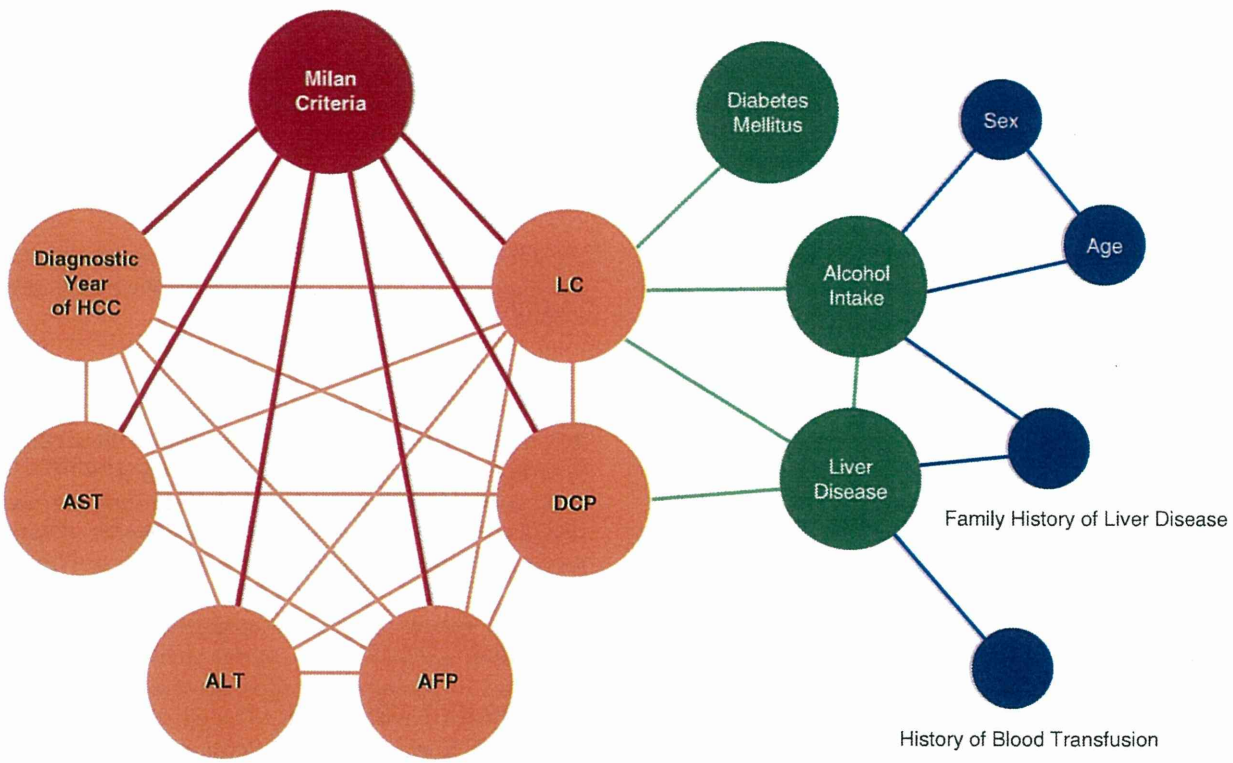

Figure 1 Graphical modeling of the interactions of the risk factors associated with the Milan criteria. AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma; LC, liver cirrhosis.

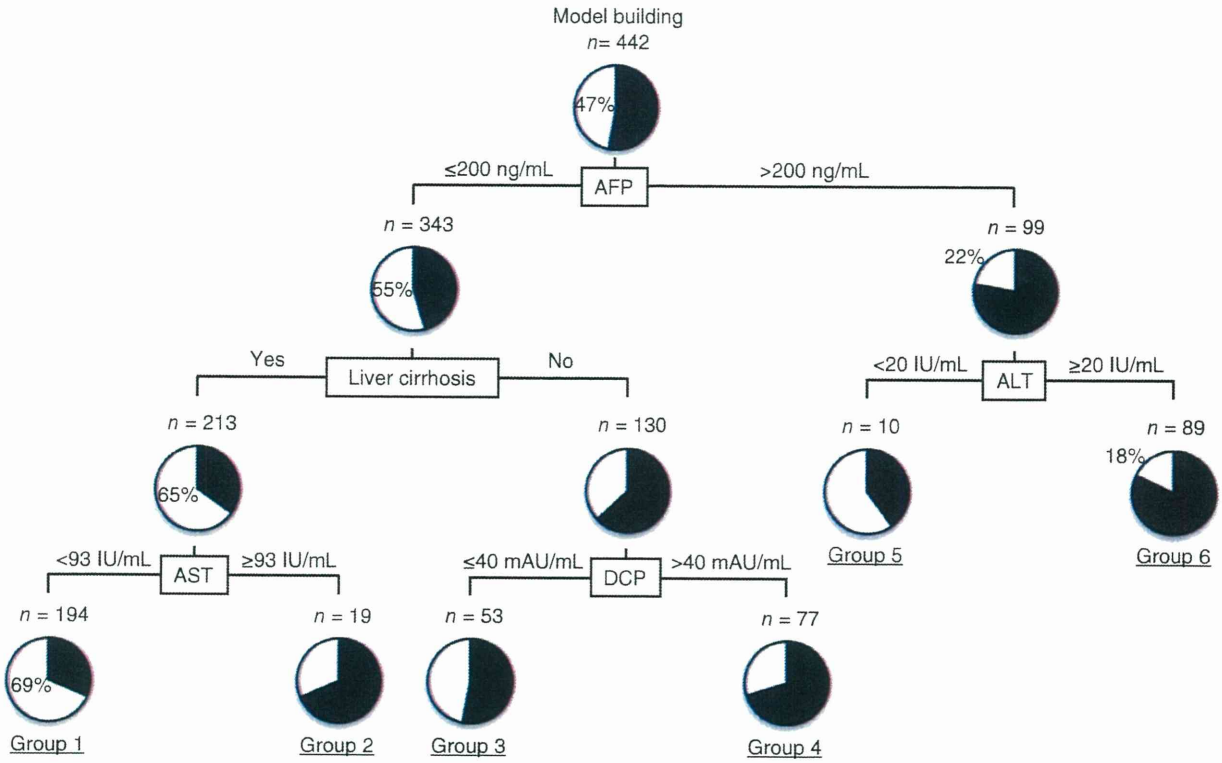

Figure 2 Decision tree algorithm of the variables associated with the Milan criteria. The patients were classified according to the indicated cut-off values of the variables. The pie graphs indicate the percentage of patients with HCC within (white)/beyond the Milan criteria in each group. AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma.

classification. Among the patients with an AFP level of 200 ng/mL or less, diagnosis of liver cirrhosis was used as the variable for the second division. Among the patients with liver cirrhosis, a serum AST level of less than 93 IU/mL was the cut-off value for the third division. Thus, 69% of the patients were within the Milan criteria, when the patients met all of the following conditions: AFP of 200 ng/mL or less; diagnosis of liver cirrhosis; and AST of less than 93 IU/mL (group 1; Fig. 2). On the other hand, only 18% of the patients were within the Milan criteria, when patients showed an AFP level of more than 200 ng/mL and an ALT level of 20 IU/mL or more (group 6; Fig. 2).

There were no significant differences in the patients' characteristics between the training dataset and the test dataset. Prediction error was obtained by applying the results of the decision tree algorithm to the test dataset. The sensitivity (proportion of patients with HCC correctly classified as beyond the Milan criteria) and specificity (proportion of patients with HCC correctly classified as within the Milan criteria) were 72.1% (75/104) and 68.4% (80/117), respectively; the overall prediction error rate was 29.8% (66/221).

DISCUSSION

IN THIS STUDY, we revealed the complex interactions of the risk factors associated with staging of NBNC-HCC using graphical modeling. In addition, we presented a decision tree algorithm to identify clinical feature profiling associated with the staging of NBNC-HCC.

Various factors seem to be intricately related to the progression of NBNC-HCC. In this study, by graphical modeling, we identified six variables directly associated with the Milan criteria: serum AST level; serum ALT level; serum AFP level; serum DCP level; diagnosis of liver cirrhosis; and diagnostic year of HCC. Chronic hepatic inflammation modulates many of the signaling cascades involved in cell proliferation, survival and invasion of

HCC.[27,28] Further, AFP and DCP are directly associated with HCC progression through the induction of cancer cell proliferation and angiogenesis, respectively.[29,30] Thus, our results are in good accordance with previous basic investigations and suggest that hepatic inflammation as well as elevated AFP and DCP levels independently accelerate the progression of NBNC-HCC.

Diagnostic year of HCC was also directly associated with the Milan criteria in this study. Although the reason for this association is unclear, a progress in serum tumor markers is a possible explanation. Because sensitivities of AFP and DCP were improved during this study period (1995–2006),[31–33] one would think that serum AFP and DCP levels are confounding factors for an association between diagnostic year of HCC and the Milan criteria.

Recently, lifestyle-related factors including alcohol intake and diabetes mellitus have been noted as risk factors for the development of NBNC-HCC.[2,10–12,34–38] Previous *in vitro* studies showed that ethanol and glucose stimulate the proliferation and migration of HCC,[39,40] indicating the direct association of alcohol intake and diabetes mellitus with NBNC-HCC progression. However, in this study, these factors were not directly associated with the Milan criteria. Although the reason for this discrepancy remains unclear, alcohol intake and diabetes mellitus were associated with the Milan criteria through diagnosis of liver cirrhosis in this study. Both ethanol consumption and diabetes mellitus can activate fibroblasts,[41,42] which are crucial components of the tumor microenvironment promoting the growth and invasion of cancer cells.[43,44] Thus, alcohol intake and diabetes mellitus may be associated with the clinical progression of NBNC-HCC through the tumor microenvironment.

Then, we created a decision tree algorithm to identify the clinical feature profiling associated with the staging of NBNC-HCC; the reproducibility of this model was confirmed by the independent validation datasets. Serum AFP level was selected for the initial classification, and serum DCP level was selected for the third division, creating groups 3 and 4. Although it is still unclear why the serum AFP level was associated with the Milan criteria to a greater extent than the serum DCP level, an association of the serum AFP level with the pathological features of HCC is a possible explanation. The AFP level is related to the number of HCC, whereas the DCP level is more specific to vascular invasion.[45–47] In this study, the staging of HCC was evaluated by using the Milan criteria, which include number and size of HCC but not vascular invasion,[26] explaining why serum AFP level was selected for the initial classification.

Diagnosis of liver cirrhosis was selected for the second division in the decision tree algorithm. Although liver cirrhosis is a well-known major risk factor for the development of HCC,[5,10,12,25,34,42] our result indicates that liver cirrhosis may suppress the progression of NBNC-HCC. We do not have any data accounting for the association between diagnosis of liver cirrhosis and suppression of the NBNC-HCC progression, the following is, however, a possible explanation for this contradiction. HCC surveillance may be performed more often in patients with liver cirrhosis than in those without liver cirrhosis,[12,25] so HCC could be identified at an early stage in patients with liver cirrhosis.

A limitation of this study is that a relationship between progression of NBNC-HCC and non-alcoholic steatohepatitis (NASH) was not evaluated. The reason is that NASH-related HCC is often diagnosed as cryptogenic cirrhosis-related HCC because of reduction of hepatic triglycerides according to the progression of NASH, so-called "burned-out NASH".[48] However, NASH is deeply involved in the development of HCC and a major reason for the increase in number of NBNC-HCC patients.[8,49,50] Recently, visceral fat accumulation is also reported to be an independent risk factor for HCC recurrence after curative treatment.[51] Thus, further study will be focused on a relationship between the progression of NBNC-HCC and NASH.

In conclusion, data mining disclosed complex associations of risk factors and clinical feature profiling associated with the staging of NBNC-HCC.

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INTERNAL MEDICINE

ORIGINAL ARTICLE

Predictive Value of the Fibrosis Scores in Patients with Chronic Hepatitis C Associated with Liver Fibrosis and Metabolic Syndrome

Hisamitsu Miyaaki, Tatsuki Ichikawa, Naota Taura, Satoshi Miuma, Hidetaka Shibata, Hajime Isomoto, Fuminao Takeshima and Kazuhiko Nakao

Abstract

Objective We evaluated patients with chronic hepatitis C (CHC) and compared the clinical and pathological features of steatosis and metabolic syndrome to identify the risk factors for CHC with severe fibrosis.

Methods One hundred seventy-one patients with biopsy-confirmed CHC were included in the study: 90 males and 81 females, age 56.2±12.8 years; 46 with obesity (BMI≥25 kg/m^2); 51 with hypertension; 36 with type 2 diabetes mellitus; and 20 with hypertriglyceridemia.

Results Steatosis was detected in 79 patients (46%); 92 patients (54%) showed no steatosis. Seventy-four patients (43%) showed mild fibrosis and 97 patients (56%) showed severe fibrosis. The variables that were significantly associated with steatosis were obesity [odds ratio 2.160 (1.010-4.727), p=0.046] and type 2 diabetes [odds ratio 3.667 (1.559-8.430), p=0.027]. The variables that were significantly associated with severe fibrosis were older age [odds ratio 2.675 (1.309-5.464), p=0.007], obesity [odds ratio 2.156 (1.006-4.619), p=0.048] and type 2 diabetes [odds ratio 8.739 (2.845-26.846), p=0.0002]. Nagasaki (N) score (the total number of specific risk factors, namely an older age, obesity, and type 2 diabetes) was higher in the severe fibrosis group than in the mild fibrosis group (mild fibrosis: severe fibrosis=1.48±1.14 vs.2.66±94, p<0.001).

Conclusion Metabolic syndrome factors, including obesity and diabetes, play a critical role in the pathogenesis of fibrosis in CHC. The N score was therefore found to be a significant predictor of severe fibrosis in CHC.

Key words: chronic hepatitis C, steatosis, liver fibrosis, type 2 diabetes mellitus, obesity

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Introduction

Hepatitis C virus (HCV) infection is one of the most common causes of chronic liver disease, affecting 2 million persons in Japan. It can lead to end-stage liver disease and hepatocellular carcinoma. Chronic HCV infection is associated with metabolic abnormalities, including insulin resistance (1-3). We recently reported that insulin resistance is associated with interferon signalling, which plays an important role in the clearance of chronic hepatitis C (CHC) during interferon therapy (4, 5). Previous studies have demonstrated the association between metabolic syndrome and hepatic fibrosis in patients with hepatitis C (6-11). Steatosis is a frequent histological finding in chronic hepatitis C virus infection, one that affects disease progression and occurrence of hepatocellular carcinoma (7, 12-15). Hepatic steatosis is associated with metabolic syndrome and non-alcoholic steatohepatitis (NASH). We previously reported that risk factors for severe fibrosis in patients with NASH were metabolic syndrome, hypertension, and, in particular, diabetes mellitus (16). In this study, we evaluated patients with chronic hepatitis C (CHC) and compared the clinical and pathological features of metabolic syndrome to identify the risk factors for CHC with severe fibrosis.

Department of Gastroenterology and Hepatology, Nagasaki University Graduate School of Biomedical Sciences, Japan
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Correspondence to Dr. Hisamitsu Miyaaki, miyaaki-hi@umin.ac.jp

Table 1. Clinical Data of the Patient Population

age	56.2 ± 12.8
Gender	male: female=90:81
HCV genotype (1:2)	123: 48
HCV viral load (Fmol/L)	5325 ± 5297
BMI (kg/m^2)	22.8 ± 2.9
BMI ≥ 25 (kg/m^2)	49 cases (29%)
Platelet (×10^4/mm^3)	15.9 ± 5.8
AST (IU/L)	72 ± 100
ALT (IU/L)	93 ± 110
Type 2 diabetes mellitus	36 cases (21%)
Hypertension	51 cases (30%)
Hyperlipidemia	20 cases (11%)

Table 2. Comparison between the Non-steatosis Group and Steatosis Group

	Steatosis n=79	Non-steatosis n=92	p value
Age (≥60 years old)	38 (48%)	45 (48%)	0.916*
Gender (male)	48 (60%)	42 (45%)	0.049*
HCV genotype 1	55 (69%)	68 (73%)	0.533*
HCV viral load (Fmol/L)	4611 ± 4872	5968 ± 5514	0.109
Platelet (×10^4/ mm^3)	15.4 ± 5.7	16.2 ± 5.7	0.324
ALT (IU/L)	106 ± 139	82 ± 77	0.173
Obesity	30(38%)	19 (20%)	0.013*
Diabetes mellitus	24 (36%)	11(12%)	0.001*
Hypertension	33 (42%)	18 (20%)	0.002*
Hypertriglycerides	13 (17%)	7 (7%)	0.070*

* χ^2 test

Methods

This retrospective study included 171 consecutive patients with biopsy-confirmed CHC who were assessed between 1996 and 2008 in Nagasaki Universities and associated hospitals. Inclusion criteria were an increased serum aminotransferase level for at least 6 months; serum anti-HCV (ELIZA; third generation); positive HCV RNA (PCR); negative serum HBs; and no other cause of liver disease, such as alcohol intake >30d/d or autoimmune or metabolic disorders (genetic hemochromatosis). Obesity was defined as a body mass index (BMI) >25 according to the World Health Organization criteria (17). Type 2 diabetes was diagnosed according to International Diabetes Federation criteria (18) (fasting glucose >110 mg/dL, or previously diagnosed type 2 diabetes). Hypertension was also diagnosed according to International Diabetes Federation criteria (18) (systolic blood pressure >130 mmHg or diastolic blood pressure >85 mmHg). Hypertriglyceridemia was diagnosed if there was documented use of anti-hypertriglyceride medications, or if fasting triglyceride levels were >150 mg/dL. All liver biopsy tissue specimens were examined using Hematoxylin-Eosin, Azan-Mallory, and silver reticulum staining. The specimens were assessed by one reviewer blinded to patient clinical and biochemical data. The diagnosis of each case was independently and histologically confirmed by liver pathologists according to the Japanese chronic hepatitis classification (New Inuyama classification) (19). Fatty change in >5% of all areas was defined as steatosis. The patients were divided into two groups based on their degree of inflammation defined as mild activity and severe activity. Fibrosis staging was scored using a 5-grade scale: F0 indicated no fibrosis, F1 was defined as fibrous portal expansion, F2 was bridging fibrosis, F3 was bridging fibrosis with lobular distortion, and F4 indicated the presence of cirrhosis. The mild fibrosis group was defined as having a score of F0-2 and severe fibrosis was defined as a score of F3 or F4.

We defined the Nagasaki (N) score as the total number of risk factors for severe fibrosis. The risk factors were age (≥ 60 years old), obesity, and type 2 diabetes. In the N score, we doubled the presence of diabetes as a factor because the estimation value of DM was 2 times higher than that of the other factors (older age and obesity) in our logistic regression formula. Therefore, we defined N score as follows (20):

N score= age (≥60 years old) +Obesity (BMI≥25) +2× diabetes mellitus

The N score was compared with other non-invasive predictors of fibrosis stage, such as the AST to platelet ratio index (APRI), and the platelet APRI was defined as follows:

APRI=AST level (/ULN)/ platelet count (10^9/L) ×100.

Statistical significance was determined by SPSS analytical software (IBM, Armonk, NY). We used Student's t-test and the chi-square test to perform analyses. A multivariate analysis was performed using binary logistic regression analysis.

Results

Among the 171 patients, 90 males and 81 females were examined. The median body mass index (BMI) was 22.8± 2.9 kg/m^2. Type 2 diabetes was diagnosed in 21% of patients, hypertension in 30%, and hyperlipidemia in 11% (Table 1).

Histological findings

Steatosis was detected in 79 patients (46%). We found mild activity in 149 patients and severe activity in 22 patients. We found no fibrosis in 18 patients (11%) (F0); mild fibrosis in 56 patients (33%) (F1); moderate fibrosis in 33 patients (19%) (F2); bridging fibrosis in 30 patients (17%) (F3); and cirrhosis in 34 patients (20%) (F4).

Steatosis

The two steatosis groups are shown in Table 2. Males were significantly more common in the non-steatosis group than in the steatosis group (60% vs. 45%, p=0.049). The patients with obesity (38% vs. 20%, p=0.013), type 2 diabetes

Table 3. Multivariate Logistic Regression Analysis of the Association of Steatosis with the Risk of Fibrosis

	odds ratio	p value
Gender (male)	1.462 (0.765~2.888)	0.263
Obesity (BMI≥ 25 kg/m^2)	2.160 (1.010~4.227)	0.046
Type 2 diabetes	3.667 (1.559~8.430)	0.027
Hypertension	2.318 (1.151~4.864)	0.002

Table 4. Comparison between Mild Fibrosis and Severe Fibrosis

	Severe fibrosis n=97	Mild fibrosis n=74	p value*
Age (≥60 years old)	56 (59%)	23(32%)	0.001*
gender (male)	51(52%)	39 (52%)	0.987*
HCV genotype 1	73 (75%)	50 (67%)	0.348 *
HCV viral load	5924 ± 5490	4864 ± 5035	0.214
Platelet (×10^4/ mm^3)	13.5 ± 4.7	19.0 ± 5.4	<0.001
ALT	104.2 ± 129.9	79.5 ± 77.7	0.126
obesity	34 (31%)	15 (20%)	0.034*
Type 2 diabetes	32 (33%)	4 (5%)	<0.001*
Hypertension	35 (38%)	16 (18%)	0.041 *
Hypertriglyceride	11 (11%)	9 (12%)	0.868 *

χ^2 test

(36% vs. 12%, p=0.001), and hypertension (42% vs. 20%, p=0.002) were significantly more likely to be in the non-steatosis group than the steatosis group. There were no significant differences between the other clinical features (age, incidence of the HCV genotype, HCV viral load, ALT, platelet count, hypertriglycemia). In a multivariate logistic regression analysis, obesity, type 2 diabetes, and hypertension were independent predictors for steatosis [odds ratio 2.160 (1.010-4.227), p=0.046, odds ratio 3.667 (1.559-8.430), p=0.027, odds ratio 2.318 (1.151-4.864), p=0.002)] (Table 3).

The incidence of severe activity was not significantly different between the two groups (non-steatosis group: steatosis group= 9.7%: 16.4%, p=0.2849).

Severe fibrosis risk factors

The risk factors of the two fibrosis groups are shown in Table 4. The prevalence of older patients (≥60 years old) in the severe fibrosis group was significantly greater than that of younger patients (32% vs. 59%, p=0.001). The prevalence of obesity, type 2 diabetes, and hypertension was significantly higher in the severe fibrosis group than in the mild fibrosis group (31% vs. 20%, p=0.034, 33% vs. 5%, p <0.001, 38% vs. 18%, p=0.041, respectively). The platelet count was significantly lower in the severe fibrosis group than in the mild fibrosis group (13.5±4.7 vs. 19.0±5.4, < 0.001). There were no significant differences between the other clinical features (age, incidence of the HCV genotype, HCV viral load, ALT, hypertriglycemia). In a multivariate logistic regression analysis, older age, type 2 diabetes, and obesity were independent predictors for severe fibrosis [coefficient 0.984 odds ratio 2.675 (1.309-5.464), p=0.007, coefficient 2.168 odds ratio 8.739 (2.845-26.846), p=0.0002, coefficient 0.768 odds ratio 2.156 (1.006-4.619), p=0.048)] (Table 5).

Table 5. A Multivariate Logistic Regression Analysis of the Association of Severe Fibrosis with Various Risk Factors

	Odds ratio	p value
Age (60 years old)	2.675 (1.309～5.464)	0.007
Obesity	2.156 (1.006～4.619)	0.048
Type 2 DM	8.739 (2.845～26.846)	0.0002
Hypertension	1.087 (0.487～2.426)	0.8394

The fibrosis stage was significantly worse in the steatosis group than in the non-steatosis group (1.78±1.28 vs. 2.34± 1.29, p=0.005).

Predictive score of severe fibrosis

The N score is the total number of risk factors, including: older age (≥60 years old), obesity, and type 2 diabetes. N score were significantly higher in the severe fibrosis group than in the mild fibrosis group (1.48±1.14 vs. 2.66±94, p< 0.001).

We found that 17 of 53 patients (32%) with an N score of 0 had severe fibrosis, 31 of 63 (49%) with an N score of 1, 25 of 29 (86%) with an N score of 2, 21 of 23 (91%) patients with an N score of 3, and 3 of 3 (100%) patients with an N score of 4 had severe fibrosis.

The ROC curve (Fig. 1) shows the respective sensitivities and specificities for any combination of 1 to 4 of the N score. An N score of 2 provides the best combination of sensitivity (0.50) and specificity (0.91) for predicting severe fibrosis.

Compared with other non-invasive predictors of significant fibrosis, the sensitivity and specificity of an APRI of 1.5 were 46% and 93%, and those for a platelet count of 12×10^4/mm^3 were 44% and 91%.

Discussion

In this study, we analyzed the correlation between metabolic syndrome and pathological findings in CHC patients, and identified the clinical risk factors for severe fibrosis.

Obesity, type 2 diabetes, and hypertension were significant risk factors for severe steatsosis and fibrosis. These comorbidities of metabolic syndrome affected steatosis and the progression of severe fibrosis in the liver. Previous data suggest a strong association between the presence of steatosis and severe fibrosis in CHC (7, 12, 13). In this study, patients in the steatosis group had more severe fibrosis than

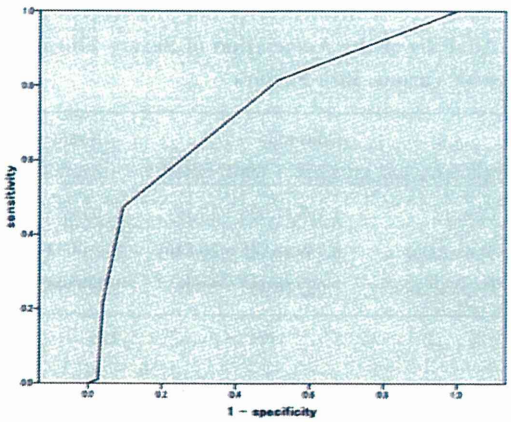

Figure 1. **ROC curve for the Nagasaki (N) score. A cut-off N score of 2 gives the sensitivity (0.50) and specificity (0.91). The area under the ROC curve is 0.732.**

Table 6. **The Incidence of Severe Fibrosis in Patients with Different N Scores**

	Severe fibrosis
N Score 0	17/53 (32%)
N Score 1	31/63 (49%)
N Score 2	25/29 (86%)
N Score 3	21/23 (91%)
N Score 4	3/3 (100%)

those in the non-steatosis group. It remains controversial whether or not hepatic steatosis may accelerate fibrosis by stimulating the activity of CHC. In this study, the ALT levels and the incidence of severe inflammation in the steatosis group were not significantly higher than in the non-steatosis group. Therefore, hepatic steatosis may not promote liver fibrosis by liver cell injury.

Evidence indicates that hepatic steatosis, which is affected by metabolic syndrome, may accelerate the progression of fibrosis in patients with CHC. The findings are consistent with those from other reports that demonstrate an association between effective weight loss and reductions in steatosis, ALT levels, and fibrosis stage in patients with CHC (21).

HCV core protein increases reactive oxygen species (ROS) and lipid peroxidation, leading to liver damage and fibrosis (22, 23). Core protein also reduces microsomal triglyceride transport protein function, leading to hepatic steatosis. Infection with the HCV virus affects liver steatosis as well as fibrosis (24). Thus, both host and viral factors induce steatosis and play a role in severe fibrosis in chronic hepatitis C.

We found a significant correlation between the severity of hepatic fibrosis and the comorbidites of metabolic syndrome, including obesity, diabetes mellitus, and hypertension. Our previous study showed that metabolic syndrome, including diabetes mellitus and hypertension, was a risk factor for severe fibrosis in patients with NASH (16). These risk factors in patients with NASH were similar to those in CHC patients, suggesting that the mechanism underlying the liver fibrosis in CHC patients resembles that of NASH.

Type 2 diabetes and obesity are correlated with insulin resistance. We previously reported that the development of liver fibrosis is associated with insulin resistance in CHC patients (25). Outcomes from the present study show the adverse effects of insulin resistance on liver fibrosis in CHC patients.

Previous data also have shown that obesity and diabetes mellitus are associated with progression of fibrosis in CHC (6, 8, 9, 26). Few prior studies have been conducted in Asian patients. The present study shows that metabolic syndrome, including obesity and diabetes, also predict severe fibrosis in Asian patients.

Taken together, although steatosis, fibrosis and metabolic syndrome seem to be associated with each other, our cross-sectional study did not identify any associations between these factors. Further studies will be necessary to confirm whether these conditions are associated, or whether they act as independent risk factors.

We defined the N score as the total number of risk factors for severe fibrosis. The risk factors were age ($\geq$60 years old), obesity, and type 2 diabetes. In the N score, we doubled the presence of diabetes as a factor because the estimation value of DM was 2 times that of other factors (older age and obesity) in our logistic regression formula (risk factor). The N score was significantly higher in the severe fibrosis group than in the mild fibrosis group. About 90% of the patients in the severe fibrosis group had an N score $\geq$2. An N score $\geq$2 indicates a high risk for severe fibrosis. Our results suggest that half of the patients in the severe fibrosis group also had metabolic disorders, including diabetes and obesity. Conversely, there was no association between metabolic syndrome and other factors.

While the specificity of the N score (0.91) is very good, the sensitivity (0.5) is not sufficient. This suggests that there are two or more mechanisms underlying the progression of fibrosis, and metabolic syndrome represents one of them.

Compared to other non-invasive markers, the sensitivity and specificity of the N score was equal to the platelet count and APRI. The N score is a simple score to calculate, and it adds together the three risk factors. Therefore, determining the N score is considered to be an easy way to predict the presence of severe liver fibrosis in CHC patients.

The present study has limitations. We cannot perform a validation due to the fact that our sample size was so small. Now, we are planning to perform a validation set to confirm the value of N score.

In conclusion, older age, obesity and type 2 diabetes are significant predictors of severe fibrosis in Japanese CHC patients. The total number of these risk factors in patients could be a useful marker for predicting severe fibrosis in pa-

tients with CHC.

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

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Living donor liver transplantation from a donor previously treated with interferon for hepatitis C virus: a case report

Masaaki Hidaka[1], Mitsuhisa Takatsuki[1], Akihiko Soyama[1], Hisamitsu Miyaaki[2], Tatsuki Ichikawa[2], Kazuhiko Nakao[2], Takashi Kanematsu[1] and Susumu Eguchi[1*]

Abstract

Introduction: Selecting a marginal donor in liver transplantation (LT) remains controversial but is necessary because of the small number of available donors.

Case presentation: A 46-year-old Japanese woman was a candidate to donate her liver to her brother, who had decompensated liver cirrhosis of unknown origin. Eight years before the donation, she had a mild liver dysfunction that was diagnosed as a hepatitis C virus (HCV) infection (serotype 2). She had received anti-viral therapy with interferon α-2b three times weekly for 24 weeks and had a sustained viral response (SVR). A biopsy of her liver before the donation showed normal findings without any active hepatitis, and her serum was negative for HCV-RNA. Only 67 patients have undergone LT from a cadaveric donor in Japan. The family in this case decided to have living donor LT. A careful selection for the liver graft donation was made; however, since she was the only candidate, we approved her as a living donor. She was discharged nine days after the liver donation. Her liver function recovered immediately. A computed tomography scan showed sufficient liver regeneration one year later. Her brother also had good liver function after LT and had no HCV infection 48 months after surgery and no *de novo* malignancy. Neither of the siblings has developed an HCV infection.

Conclusions: A patient with SVR status after interferon therapy might be considered a candidate for living donor LT but only if there are no other possibilities of LT for the recipient. A careful follow-up of the donor after donation is needed. The recipient also must have a very close follow-up because it is difficult to predict what might happen to the graft with post-transplant immunosuppression.

Introduction

The number of deceased donor liver transplantations (DDLTs) in Japan is extremely small. There were 67 cases between February 1999 and January 2010, according to the Japan Organ Transplant Network [1]. Therefore, living donor liver transplantation (LDLT) is the most frequent treatment option for patients with end-stage liver disease in Japan. The main advantage of LDLT over DDLT is that the donor can be completely evaluated, before the operation, to exclude many medical problems. However, the indications for a living donor should be strict and the risk to the donor must be avoided with the greatest care. Donors with possibly morbid liver conditions, including fatty infiltration or a history of viral hepatitis, and older donors offer "marginal grafts", which should be used only after very careful evaluation. A hepatitis B virus (HBV) core antibody seropositive donor can be accepted as long as HBV surface antigen is seronegative and anti-viral treatment is administered to the recipient after transplantation [2,3]. In this way, donor safety also is established, according to several reports of this type of case [4-6]. Some investigators reported that the patients obtained a sustained viral response (SVR) after interferon therapy showed that there was no tendency to develop fibrotic liver in the future [7,8]. HCV-RNA was not detected in 88% of the serum and liver biopsies of patients with an SVR [9]. The infection rate of the recipient from an HCV-

* Correspondence: sueguchi@nagasaki-u.ac.jp
[1]Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan
Full list of author information is available at the end of the article