ORIGINAL ARTICLE

Histopathology of type C liver disease for determining hepatocellular carcinoma risk factors

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Received: January 22, 2013   Revised: June 6, 2013
Accepted: June 19, 2013
Published online: August 14, 2013

Abstract

AIM: To evaluate the histopathological findings of type C liver disease to determine risk factors for development of hepatocellular carcinoma (HCC).

METHODS: We studied 232 patients, who underwent liver biopsy for type C chronic liver disease between 1992 and 2009, with sustained virological response (SVR) after interferon therapy. The patients were divided into two groups according to the F stage 0 + 1 + 2 group (*n* = 182) and F3 + 4 group (*n* = 50). We prospectively observed and compared the incidence of HCC of the patients with SVR in the F0 + 1 + 2 and F3 + 4 groups. Then, the background factors and liver histopathological findings, including the degree of fibrosis, F stage, inflammation, necrosis, bile duct obstruction, fat deposition, and degree of irregular regeneration (IR) of hepatocytes, were correlated with the risk of developing HCC.

RESULTS: HCC developed in three of 182 (1.6%) patients in the F0 + 1 + 2 group, and four of 50 (8.0%) in the F3 + 4 group. The cumulative incidence of HCC in the former group was found to be significantly lower than in the F3 + 4 group (log rank test *P* = 0.0224). The presence of atypical hepatocytes among IR of hepatocytes in the F3 + 4 group resulted in a higher cumulative incidence of HCC, and was significantly correlated with risk of HCC development (RR = 20.748, 95%CI: 1.335-322.5, *P* = 0.0303).

CONCLUSION: Atypical hepatocytes among the histopathological findings of type C liver disease may be an important risk factor for HCC development along with progression of liver fibrosis.

Core tip: To evaluate the histopathological findings of type C liver disease to determine risk factors for the development of hepatocellular carcinoma (HCC), we studied 232 patients, who underwent liver biopsy, with sustained virological response after interferon therapy. We investigated in detail the histopathological findings, and analyzed the findings to determine the risk factors. Consequently, atypical hepatocytes among irregular regeneration of hepatocytes may be an important risk factor for HCC development, along with progression of liver fibrosis. Clarification of atypical hepatocytes as a risk factor of carcinogenesis may aid in the early diagnosis of HCC.

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INTRODUCTION

At our facility, we maintain a database of hepatitis patients who were recruited with informed consent from the 1990s. We previously reported the natural progression of patients with type C chronic hepatitis (CH) and liver cirrhosis (LC) - both attributable to hepatitis C virus (HCV) infection - over the course of about 30 years. The incidence of hepatocellular carcinoma (HCC) among those with LC was 7% per annum[1,2]. Many other studies have reported the progression of liver disease and the risk of development of HCC with HCV infection[3-8].

In this study, we collected liver biopsy samples from type C chronic liver disease patients before interferon (IFN) therapy and examined in detail the histopathological findings of the liver tissue. According to the progression of liver fibrosis (F stage), we examined the factors that may contribute to the development of HCC. We further studied the association of the condition of the hepatocytes, present during regeneration of the liver parenchyma destroyed by infection, with the incidence of HCC. The irregular regeneration (IR) of hepatocytes, which occurs as a result of repeated cycles of necrosis and regeneration of the liver parenchyma in CH, was found to be important for the prognosis of HCC[9-15]. A few studies have sought to determine the association between the histopathological findings from liver biopsies and the risk of developing HCC[4]. Therefore, patients with type C chronic liver disease who underwent liver biopsy were investigated in detail for findings on the sites and degree of fibrosis and inflammation, necrosis, IR of hepatocytes, and fat deposition in the liver. These findings were analyzed to determine the risk factors for development of HCC.

MATERIALS AND METHODS

Study population

In this study, samples from 482 patients with type C liver disease who underwent liver biopsy between 1992 and 2009 were collected prior to the start of IFN therapy. Two hundred and thirty-two patients achieved a sustained virological response (SVR) to IFN therapy. We determined the F stage in the liver (stages 0-4, as described for histological scoring) by liver biopsy according to the method of Desmet *et al*[16], Knodell *et al*[17] and Ishak *et al*[18]. Then, 232 patients were divided into two groups according to the F stage: stages 0, 1 and 2 (F0 + 1 + 2 group, *n* = 182) and stages 3 and 4 (F3 + 4 group, *n* = 50). We prospectively observed and compared the incidence of HCC in the patients with SVR in the F0 + 1 + 2 and F3 + 4

groups. The observation period was from the time of diagnosis of SVR to the time of the final examination or of diagnosis of HCC. Then, the clinical background factors and the histopathological findings at the liver biopsy were correlated with the risk of developing HCC together with progressive liver fibrosis.

All of the patients were positive for serum HCV antibody (2nd generation ELISA; Dinabot, Tokyo, Japan) and HCV RNA in serum, and negative for serum hepatitis B surface antigen (HBsAg ELISA; Dinabot), anti-nuclear antibody (indirect immunofluorescence assay: IF, Special Reference Laboratory, Tokyo, Japan), anti-smooth muscle antibody (IF), and anti-mitochondrial antibody (IF). No heavy drinkers (more than 30 g of ethanol intake daily) were included.

We confirmed the positivity and measured the concentration of HCV RNA in the blood samples using the competitive reverse transcriptase-polymerase chain reaction and DNA probe methods (Special Reference Laboratory, Tokyo, Japan) or Amplicor monitoring method (Amplicor HCV Monitor, Roche Diagnostic K.K., Tokyo, Japan). Subjects without HCC were confirmed by abdominal computed tomography (CT) or ultrasonography within the 6 mo prior to initial liver biopsy. At the time of liver biopsy, we determined the patient's body mass index, serum aspartate aminotransferase (AST) level (U/L), serum alanine aminotransferase (ALT) level (U/L), serum γ-glutamyltransferase (GT) level (U/L), serum platelet count ($\times 10^4/\mu$L), and HCV genotype, and carried out a histopathological examination of the biopsy specimens. We recorded the patient's sex and age, period of observation (years), and incidence of HCC. All patients gave their consent to be included in this study according to the Declaration of Helsinki.

Administration of IFN

Initial IFN therapy varied according to the time period when the patients were treated. From 1992 to 2001, IFN monotherapy was administered using natural (n) IFN (n-IFNα: Sumiferon, Sumitomo Pharma Co., Osaka, Japan) in 155 cases, recombinant (r)-α2a (r-IFNα2a: Canferon, Takeda Pharmaceutical Co., Osaka, Japan) in 44 cases, and r-α2b (r-IFNα2b：Intron, Schering-Plough Co., Osaka, Japan) in 121 cases. From 2001 to 2007, we treated 68 patients with combination therapy of IFN-α2b and ribavirin (Rebetol; Schering-Plough Co.). From 2007 to 2009, we administered combination therapy of Peg-IFN-α2b (Peg Intron; Schering-Plough Co.) and ribavirin in 93 cases. In accordance with the protocol based on the medical insurance system in Japan, considering the HCV genotype, we administered IFN therapy for 6-12 mo. The following classification of outcomes was determined according to the effects of IFN therapy: SVR was achieved in patients who became negative for serum HCV RNA for > 6 mo after termination of IFN therapy.

Liver biopsy

Liver tissues were taken from patients by percutaneous

needle biopsy (MONOPTY, 14 or 16 gauge; C. R. Bard Inc., Tempe, AZ, United States) with the aid of an ultrasonic echo guide within the 6 mo preceding the start of IFN therapy. These tissue specimens were fixed in 2.5% formalin, embedded in paraffin, sectioned at 3-4 μm, and stained with hematoxylin and eosin (HE).

Histological scoring

The tissues obtained by liver biopsy were evaluated for the sites and degree of fibrosis, inflammatory reaction, and necrosis, as well as the degree of IR of hepatocytes, bile duct obstruction, and fat deposition in the liver.

The histopathological findings were scored using 5 grades: score 0, none of the above features; score 1, minimal detection (observed in less than one-third of the field); score 2, mild (observed in one-third to less than two-thirds of the field); score 3, moderate (observed in two-thirds or more of the field), and score 4, severe (diffusely in all fields). The samples were scored blindly by two pathologists (Moriyama and the author), and the final value was calculated as the average of their scores (mean ± SD).

Only minimal fibrosis was observed in the portal areas of normal livers. Pericellular fibrosis was defined as fibrosis around the hepatocytes, perivenular fibrosis as fibrosis around the central vein, and portal sclerosis as fibrosing expansion of the portal area. The degree of pericellular fibrosis, perivenular fibrosis, and portal sclerosis were evaluated and assigned a score from 0 to 4 as described above for histopathological scoring. According to the method of Desmet *et al*[16] and Knodell *et al*[17], we determined the F stage in the liver (stages 0-4, as described above for histopathological scoring), including bridging fibrosis.

In evaluating hepatocellular necrosis, focal necrosis was defined as necrosis of several hepatocytes with infiltration of inflammatory cells such as macrophages and lymphocytes. Bridging necrosis refers to necrosis connecting areas, such as two portal tracts, leading to extensive hepatocellular necrosis and a state of acute hepatic failure. The patients were further defined as with (Y) or without bridging necrosis (N).

Infiltration of lymphocytes is a characteristic of viral hepatitis. The degree of inflammatory cell infiltration into the liver parenchyma (parenchymal infiltration) and portal area (periportal infiltration) were assessed, and assigned a score from 0 to 4 as described above for histopathological scoring. The degree of lymphocytic infiltration into the entire hepatic lobule (lymphoid reaction) and portal area (portal lymphocyte infiltration) was also evaluated and assigned a score from 0 to 4 as described above for histopathological scoring.

We also investigated the histopathological abnormalities characteristic of IR of hepatocytes (Figure 1), which were evaluated in each sample according to the criteria by Uchida[9], Shibata *et al*[10], Ueno *et al*[11] and Moriyama *et al*[12]. IR comprises the following six elements: dysplastic change, map-like distribution, bulging, oncocytes, nodularity, and atypical hepatocytes. Based on these, we examined the liver biopsy samples for: presence and degree of dysplastic change defined as anisocytosis characterized by variability

of the cells; presence and degree of map-like distribution, which is defined as distinct populations of hepatocytes with a homogeneous appearance within each population and separated from each other by a sharp outline; presence and degree of bulging, which is defined as expansive proliferation of hepatocytes compressing the surrounding parenchyma; presence of oncocytes with nonuniformity of the cytoplasm; presence and degree of nodality, defined as nodular appearance of hepatocytes; and presence of atypical hepatocytes characterized by cellular degeneration. Each finding of IR was evaluated and assigned a score from 0 to 4 as described above for histopathological scoring.

Bile duct obstruction was investigated according to the degree of cholangitis by lymphocytes (bile duct damage) and assigned a score from 0 to 4 as described above for histopathological scoring. Finally, the degree of fat deposition in liver (steatosis) was assessed according to the method by Brunt *et al*[19] and Matteoni *et al*[20], and assigned a score from 0 to 4 as described above for histopathological scoring.

Evaluation of the long-term outcome of patients

Patients with IFN therapy underwent abdominal ultrasonography every 3 or 6 mo and abdominal CT examination every 6-12 mo to check for the occurrence of HCC. When space occupying lesions (SOLs) were detected in the livers of the patients by dynamic CT, enhancement of SOLs was observed in the early phase of dynamic CT and the disappearance of SOL staining was observed in the late phase. A precise diagnosis was made by abdominal angiography. When SOLs in the liver were not enhanced in the early phase of dynamic CT, or if a precise diagnosis could not be made by abdominal angiography, tumor biopsy was carried out and a precise diagnosis was made on the basis of the pathological findings.

Statistical analysis

Sex, genotype, and bridging necrosis (Y/N) were compared using the χ^2 test for independence. The remaining parameters including the clinical characteristics at the time of liver biopsy and the liver histopathological findings are shown as mean ± SD and were compared using the Mann-Whitney *U* test. Cumulative incidence curves were determined with the Kaplan-Meier method and the differences between groups were assessed using the log-rank test. Analysis of risk factors for HCC occurrence was made using the Cox proportional hazard model and these were compared by multivariate analysis. These were performed using SPSS 11.0 software (SPSS Inc., Chicago, IL, United States). *P* < 0.05 was considered significant.

RESULTS

Clinical background factors at time of liver biopsy and liver histopathological findings in SVR patients according to F stage

Comparison of clinical background factors at the time of liver biopsy and liver histopathological findings in
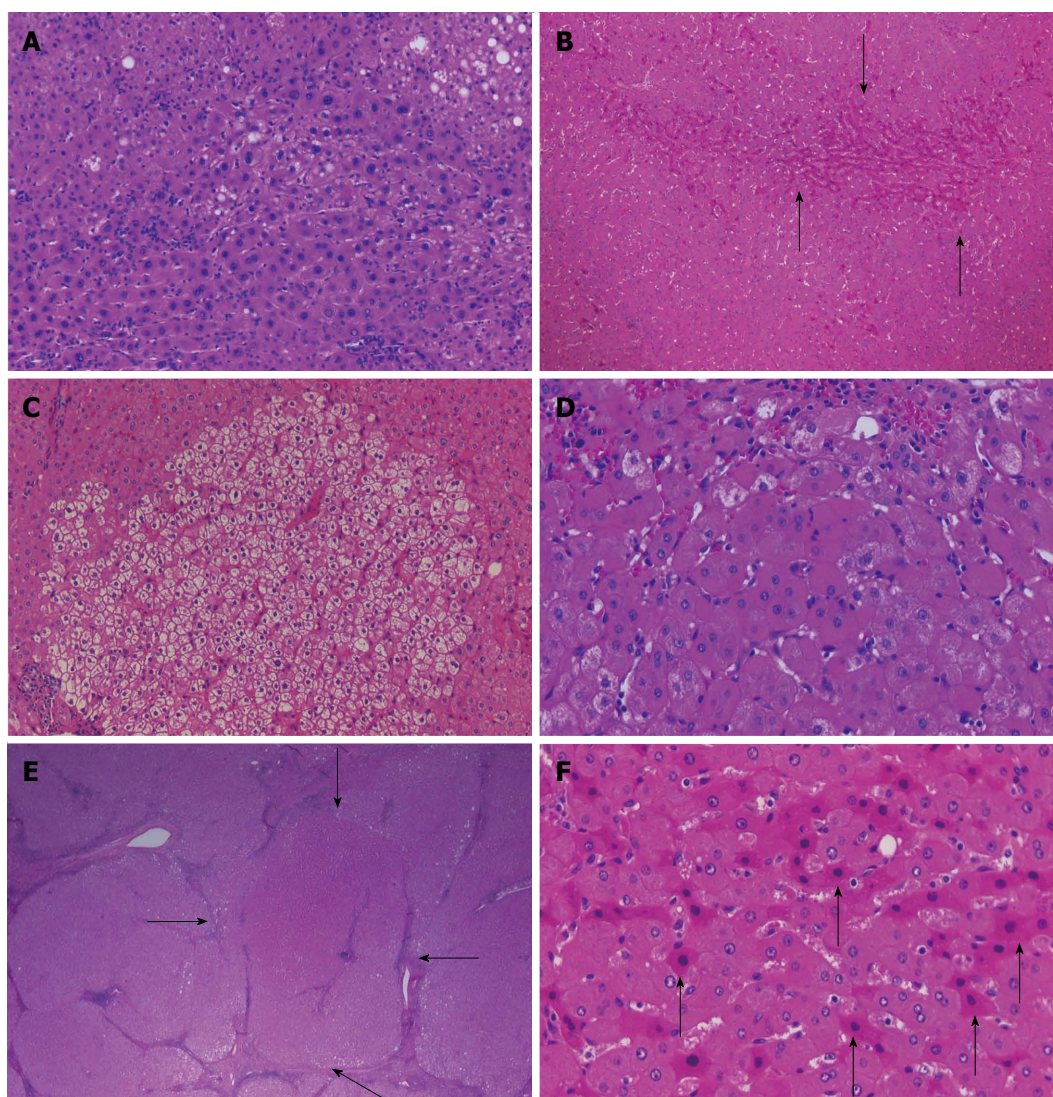
Figure 1 Features of irregular regeneration of hepatocytes. Microscopic views are shown of the biopsied liver tissues (F1 and F3 stage) of representative patients with hepatitis C virus infection. A: Dysplastic change; anisocytosis characterized by variability of cell size with focal dysplastic change [hematoxylin and eosin (HE); × 200]; B: Map-like distribution; distinct populations of hepatocytes with a homogeneous appearance within each population are separated from each other by a sharp outline (arrows) (HE; × 100); C: Bulging; expansive proliferation of hepatocytes compressing the surrounding parenchyma (HE; × 100); D: Oncocytes; oncocytic change of hepatocytes (HE; × 400); E: Nodularlity; nodular arrangement of the parenchyma (arrows) (HE; × 40); F: Atypical hepatocytes; degeneration of hepatocytes (arrows) (HE; × 400). These histopathological findings were scored using 5 grades: score 0, none; score 1, minimal (observed in less than one-third of the field); score 2, mild (observed in one-third to less than two-thirds of the field); score 3, moderate (observed in two-thirds or more of the field) and score 4, severe (diffusely in all fields). The findings were scored as the average (mean ± SD).

SVR patients according to the F stage (F0 + 1 + 2, n = 182 and F3 + 4, n = 50) was performed (Tables 1 and 2). As demonstrated in Table 2, the number of patients for each score was compared, then the presence of bridging necrosis (Y/N) was compared, and the remaining parameters are presented as mean ± SD. The findings demonstrated that there was a higher level of deterioration of the liver in the F3 + 4 group as compared to the F0 + 1 + 2 group; liver fibrosis was more progressive, age, AST, ALT, and α-fetoprotein (AFP) were higher, platelet count was lower, and inflammatory reaction was stronger.

Cumulative incidence of HCC between patients with SVR in the F0 + 1 + 2 and F3 + 4 groups
The cumulative incidence of HCC was compared be-tween patients with SVR in the F0 + 1 + 2 and F3 + 4 groups by the Kaplan-Meier method (Figure 2A). HCC developed in seven of 232 (3.0%) patients, which included three of 182 (1.6%) patients in the F0 + 1 + 2 group, and four of 50 (8.0%) patients in the F3 + 4 group. The cumulative incidence of HCC in the former group was found to be significantly lower than in the F3 + 4 group (log rank test P = 0.0224).

Cumulative incidence of HCC in the F0 + 1 + 2 and F3 + 4 patients with or without atypical hepatocytes
Among the histopathological findings in the liver, the occurrence of the IR of hepatocytes is analyzed to determine the risk factors for development of HCC together with progressive liver fibrosis. In order to correct for the
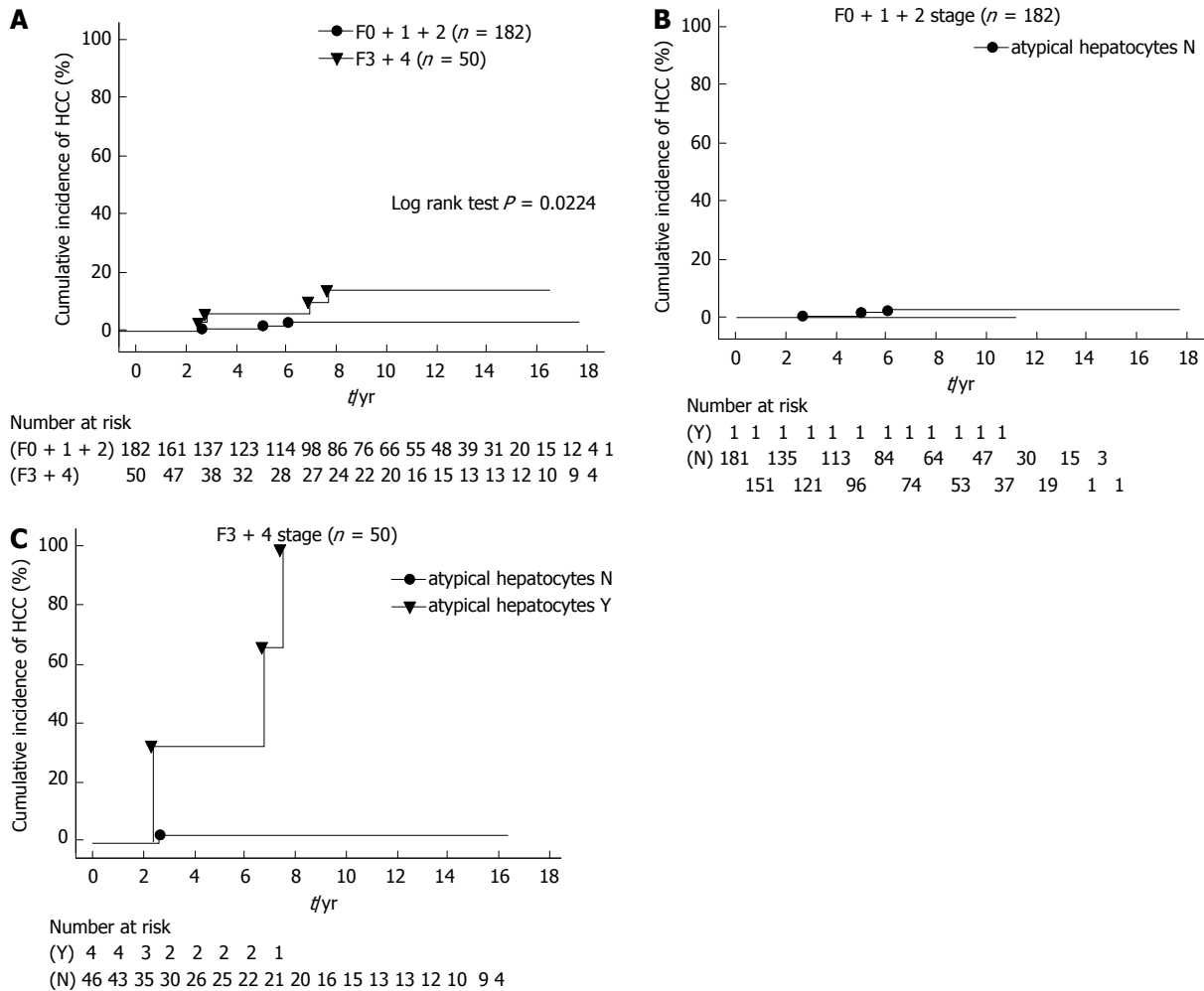
A: F0 + 1 + 2 (*n* = 182), F3 + 4 (*n* = 50). Log rank test *P* = 0.0224

Number at risk
(F0 + 1 + 2) 182 161 137 123 114 98 86 76 66 55 48 39 31 20 15 12 4 1
(F3 + 4)   50 47 38 32 28 27 24 22 20 16 15 13 13 12 10 9 4

B: F0 + 1 + 2 stage (*n* = 182), atypical hepatocytes N

Number at risk
(Y) 1   1   1   1   1   1   1   1   1   1   1   1
(N) 181 135 113 84 64 47 30 15   3
             151 121 96 74 53 37 19   1   1

C: F3 + 4 stage (*n* = 50), atypical hepatocytes N, atypical hepatocytes Y

Number at risk
(Y) 4   4   3   2   2   2   2   1
(N) 46 43 35 30 26 25 22 21 20 16 15 13 13 12 10 9 4

Figure 2 Comparison of the cumulative incidence of hepatocellular carcinoma. A: Between patients with sustained virological response in the F0 + 1 + 2 and F3 + 4 groups. The cumulative incidence of hepatocellular carcinoma (HCC) was compared between patients with sustained virological response in the F0 + 1 + 2 and F3 + 4 groups by the Kaplan-Meier method. HCC developed in seven of 232 (3.0%) patients, which included three of 182 (1.6%) patients in the F0 + 1 + 2 group, and four of 50 (8.0%) patients in the F3 + 4 group. The cumulative incidence of hepatocellular carcinoma in the former group was found to be significantly lower than in the F3 + 4 group (log rank test *P* = 0.0224). Values below the graphs show the numbers at risk; B and C: Between patients with sustained virological response in the atypical hepatocytes (N) and (Y) groups, according to the F0 + 1 + 2 and F3 + 4 groups. HCC developed in seven of 232 (3.0%) patients, of which three belonged to the F0 + 1 + 2 group and four to the F3 + 4 group. To correct for the atypical hepatocytes, the 232 patients were divided into two groups as follows: those with an absence (score 0) of atypical hepatocytes (N group, *n* = 227) and those with presence (score 1) of atypical hepatocytes (Y group, *n* = 5). The Y group included all cases with scores 1-4, but actually there were only cases with score 1. The cumulative incidence of HCC was compared in the F0 + 1 + 2 and F3 + 4 patients according to the atypical hepatocyte status by the Kaplan-Meier method. For the F3 + 4 patients, we found that there was a significantly lower cumulative incidence of HCC in the N group as compared to the Y group (log rank test, *P* = 0.0001). Values below the graphs show the numbers at risk.

IR of hepatocytes, dysplastic change, map-like distribution, bulging, oncocytes, nodularlity, and atypical hepatocytes, the 232 patients were divided into two groups as follows: those with an absence (score 0) of IR of hepatocytes (N group) and those with presence (score 1-4) of IR of hepatocytes (Y group) according to the F0 + 1 + 2 and F3 + 4 groups. The cumulative incidence of HCC was compared with the IR of hepatocytes according to the F0 + 1 + 2 and F3 + 4 patients by the Kaplan-Meier method.

For example, to correct for the atypical hepatocytes, the 232 patients were divided into two groups as follows: those with an absence (score 0) of atypical hepatocytes (N group, *n* = 227) and those with presence (score 1-4) of atypical hepatocytes (Y group, *n* = 5). The Y group included all cases with score 1, but there were only cases

with score 1. For the F3 + 4 patients, we found that there was a significantly lower cumulative incidence of HCC in the N group as compared to the Y group (log rank test, *P* = 0.0001, Figure 2B and C). However, there was no significant difference in the remaining IR of hepatocytes (data not shown). Thus, our findings demonstrate that with the progression of liver fibrosis, those patients with atypical hepatocytes among the IR of hepatocytes have a higher cumulative incidence of HCC.

Comparison of clinical background factors to determine association with development of HCC

In the F0 + 1 + 2 and F3 + 4 groups, the findings were analyzed to determine the risk factors for development of HCC using the Cox proportional hazard regression method. We compared the clinical background factors at

Table 1 Comparison of clinical background factors at the time of liver biopsy in sustained virological response patients, between F0 + 1 + 2 and F3 + 4 groups *n* (%)

Parameter		F0 + 1 + 2	F3 + 4	P value
Number	232	182	50	
Gender (male)	136 (58.6)	104 (57.1)	32 (64.0)	0.3832
Age (yr)	47.9 ± 12.2	45.9 ± 12.4	55.2 ± 8.3	0.0001
BMI	22.6 ± 3.3	22.7 ± 3.3	22.2 ± 3.6	0.5466
AST (U/L)	55.2 ± 45.9	49.8 ± 46.6	74.5 ± 37.6	0.0007
ALT (U/L)	75.5 ± 62.7	70.5 ± 63.0	93.5 ± 58.9	0.0216
γ-GT (U/L)	62.3 ± 71.8	61.4 ± 76.7	65.8 ± 50.4	0.7048
Platelet count ($\times 10^4/\mu$L)	18.8 ± 6.2	20.2 ± 6.0	13.9 ± 3.9	0.0001
Genotype type 1/2	108 (46.5)/ 124 (53.5)	86 (47.2)/ 96 (52.8)	22 (44.0)/ 28 (56.0)	0.6830
AFP (ng/mL)	7.7 ± 13.3	5.4 ± 8.6	15.5 ± 21.4	0.0008
Observation period (yr)	7.5 ± 4.9	7.4 ± 4.7	8.0 ± 5.5	0.4350

These 232 patients were divided into two groups according to the F stage: stages 0, 1, and 2 (F0 + 1 + 2 group, *n* = 182) and stages 3-4 (F3 + 4 group, *n* = 50). Comparison of clinical background factors at the time of liver biopsy in SVR patients according to the F stage (F0 + 1 + 2 and F3 + 4) was performed, and the data are shown. The clinical background factors demonstrated that there was a higher level of deterioration of the liver in the F3+4 group as compared to the F0 + 1 + 2 group; Age, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and α-fetoprotein (AFP) was higher, and platelet count was lower. The parameters are presented as mean ± SD. AST: Upper limit of the normal range is 38 U/L; ALT: Upper limit of the normal range is 44 U/L; γ-glutamyltransferase (GT): Upper limit of normal is 73 U/L; AFP: Upper limit of normal is 20.0 ng/mL; Platelet count: reference value is $15.0 \times 10^4 - 35.0 \times 10^4/\mu$L. BMI: Body mass index.

liver biopsy of the patients with SVR in the F0 + 1 + 2 and F3 + 4 groups (Table 3). Univariate analysis using the Cox proportional hazard regression method was applied to these parameters to determine the clinical background factors that were associated with the development of HCC. None of the clinical background factors showed any significant associations with HCC. Blank values in the table signify that the bias was applied, but could not be statistically processed.

Comparison of liver histopathology to determine factors associated with development of HCC

We compared the histopathological findings of liver biopsy in patients with SVR in the F0 + 1 + 2 and F3 + 4 groups (Table 3). Univariate analysis using the Cox proportional hazard regression method was applied to these parameters to determine the liver histopathological findings that were associated with the development of HCC. Significant correlations were found for map-like distribution (RR = 3.082, 95%CI: 1.066-8.913, *P* = 0.0378) and atypical hepatocytes (RR = 42.055, 95%CI: 4.303-411.0, *P* = 0.0013) in the F3 + 4 patients. In the F0 + 1 + 2 patients, none of the liver histopathological findings showed any significant associations with HCC. Blank values in the table signify that the bias was applied, but could not be statistically processed.

Multivariate analysis using the Cox proportional hazard regression method was applied to the parameters that

could be of statistical significance in Table 3 in each of the F stage groups, and the findings are shown in Table 4. In the F0 + 1 + 2 group, none of the factors showed any significant correlation with the development of HCC. In the F3 + 4 group, map-like distribution (RR = 1.687, 95%CI: 0.404-7.053, *P* = 0.4734) was not found to have any correlation while the presence of atypical hepatocytes (RR = 20.748, 95%CI: 1.335-322.5, *P* = 0.0303) was shown to be significantly associated with HCC development.

DISCUSSION

In Japan, $\geq$ 90% of cases of LC associated with HCC are attributable to hepatitis B or C virus infection. Many studies of hepatitis C have reported risk factors for HCC other than the virus, such as the progression of fibrosis, male sex, increasing age, consumption of a large amount of alcohol, and excess iron in the liver[7,21-23]. It has been reported that the occurrence of HCC may be suppressed by reducing the degree of inflammation, regardless of the presence or absence of HCV[24-26]. Furthermore, among the liver histopathological findings, it has been reported that the progression of liver fibrosis (F stage) constitutes a risk factor for carcinogenesis[4]. Therefore, we sought to identify the liver histopathological findings, other than the F stage, that are correlated with the risk of carcinogenesis. Previously, we have reported that the degree of IR is related to factors other than liver fibrosis that contribute to liver carcinogenesis. It has been reported that the cumulative incidence of HCC was significantly lower in cases in which the degree of IR had improved after IFN therapy than in cases without IFN therapy[11,12]. If the degree of inflammation and necrosis in the liver were also improved by IFN therapy, this would be reflected by an improvement in the histopathological findings, particularly the degree of IR and liver fibrosis, and by a reduction in the incidence of HCC. Moreover, it is generally accepted that HCV may be a direct cause of HCC[27]. Thus, by considering the differences in liver histopathological findings in patients with SVR following viral clearance, it was possible to eliminate the direct effects of the virus on HCC progression. If the degree of inflammation and necrosis in the liver was improved following elimination of the virus by IFN therapy, the development of HCC would be attributable, at least in part, to nonviral factors. Therefore, we investigated the risk factors in detail, including the degree of liver IR and the histopathological findings of liver biopsy specimens, and prospectively examined the findings separately according to the degree of liver fibrosis in SVR patients.

In type C chronic disease, it is said that active inflammation persists in the liver, with the formation of lymphoid follicles, varying degrees of liver parenchyma failure, and IR of hepatocytes, ultimately leading to the formation of small regenerative nodules. In other words, IR of hepatocytes, which occurs in the liver as a result of repeated cycles of necrosis and regeneration, is recognized as a population of hepatocytes that differ from normal ones in size, appearance, and arrangement of

Table 2 Comparison of liver histological findings at the time of liver biopsy in sustained virological response patients, between F0 + 1 + 2 and F3 + 4 groups

Parameter	Score					F0 + 1 + 2	F3 + 4	*P* value
	0	1	2	3	4			
Irregular regeneration								
Dysplastic change	109	50	49	21	3	0.769 ± 0.998	1.660 ± 1.081	0.0001
Oncocytes	211	16	4	1	0	0.066 ± 0.290	0.300 ± 0.647	0.0003
Map-like distribution	160	37	22	13	0	0.396 ± 0.756	0.960 ± 1.142	0.0001
Nodular arrangement	216	13	1	2	0	0.027 ± 0.164	0.320 ± 0.713	0.0001
Bulging	206	18	6	2	0	0.104 ± 0.400	0.340 ± 0.688	0.0022
Atypical hepatocytes	227	5	0	0	0	0.005 ± 0.074	0.080 ± 0.274	0.0012
Inflammatory cell infiltration								
Peri-portal	0	65	127	35	5	1.819 ± 0.652	2.260 ± 0.828	0.0001
Parenchymal	0	37	149	44	2	1.978 ± 0.585	2.300 ± 0.678	0.0010
Portal lymphocyte	0	5	103	121	3	2.456 ± 0.562	2.780 ± 0.507	0.0003
Portal lymphoid reaction	0	6	47	78	101	3.132 ± 0.850	3.360 ± 0.802	0.0904
Fibrosis								
F stage	5	128	49	34	16			
Portal sclerosis	113	81	32	6	0	0.615 ± 0.783	1.020 ± 0.795	0.0014
Pericellular fibrosis	89	90	41	11	1	0.802 ± 0.870	1.260 ± 0.853	0.0011
Perivenular fibrosis	63	66	65	25	0	1.236 ± 1.016	1.243 ± 0.895	0.9691
Bridging necrosis Y (presence)						0/228	4/228 (1.7%)	0.0020
Bile duct damage	123	71	26	9	2	0.597 ± 0.861	1.000 ± 0.904	0.0041
Steatosis	96	46	68	18	1	0.944 ± 0.990	1.429 ± 1.118	0.0035

Comparison of liver histological findings in sustained virological response patients according to the F stage (F0 + 1 + 2 and F3 + 4) was performed, and the data are shown. The histological findings demonstrated that there was a higher level of deterioration of the liver in the F3 + 4 group as compared to the F0 + 1 + 2 group; liver fibrosis was more progressive and inflammatory reaction was stronger. The number of patients for each score was compared, then the presence of bridging necrosis (Y/N) was compared and the remaining parameters are presented as mean ± SD. Scoring: score 0, none; score 1, minimal (observed in less than one third of the field); score 2, mild (observed in one third to less than two thirds of the field); score 3, moderate (observed in two thirds or more of the field) and score 4, severe (diffusely in all fields).

the nucleus. As a result, nodular lesions often are detectable in the livers of patients with type C chronic disease. These nodular lesions may be classified into two types: dysplastic nodules and early HCC. In addition, dysplastic nodules are divided into low-grade and high-grade dysplastic nodules or borderline lesions[28]. Fatty changes may be seen in 40% of high-grade dysplastic nodules, but are not observed in the low-grade ones, and regenerative large nodules are observed at high frequency in early HCC[29,30].

We compared the cumulative incidence of HCC between patients with low (F0 + 1 + 2) and high (F3 + 4) degree of liver fibrosis, and it was found that the former group had a significantly lower rate than the F3 + 4 group, suggesting that as fibrosis progresses in the liver, HCC is more likely to occur. It is clear that liver fibrosis progresses and also contributes to an increased risk of the development of HCC. In this study, factors associated with the development of HCC were identified by comparing patients with SVR by multivariate analysis using the Cox proportional hazard regression method, which was applied to the clinical and histopathological parameters. We found that among all the investigated factors, the presence or absence of atypical hepatocytes among IR of hepatocytes may be an important risk factor for HCC development along with progression of liver fibrosis.

Moreover, patients with progressive liver fibrosis and atypical hepatocytes among the IR of hepatocytes also had a higher cumulative incidence of HCC. Therefore, our results clearly demonstrate the occurrence of atypical hepatocytes in progressive liver fibrosis as a risk factor of HCC. This finding is also considered important for determination of a patient's therapeutic options.

HCC, stemming from LC induced by HCV infection, and other nonviral liver diseases, such as nonalcoholic steatohepatitis (NASH)[31-33] and autoimmune hepatitis[34], may develop through carcinogenic mechanisms based on inflammation[35,36]. Accordingly, we consider that the atypical hepatocytes may also contribute to the development of HCC in these nonviral diseases. Thus, during the assessment of the histopathological findings from liver biopsies, attention should be paid to the liver fibrosis, and additionally the presence or absence of atypical hepatocytes according to the type of liver disease.

In conclusion, among the histopathological findings in the liver of type C chronic disease, the occurrence of atypical hepatocytes in the IR of hepatocytes is significantly correlated with the risk of developing HCC together with progressive liver fibrosis. We believe that clarification of this finding as a risk factor of carcinogenesis may aid in the early diagnosis of HCC, and it would be meaningful to perform liver biopsy in patients with progression of liver fibrosis. Thus, by treating patients for both hepatitis C infection and atypical hepatocytes, we may attain an increase in the survival rate and a lower incidence of HCC.

Table 3 Factors associated with the development of hepatocellular carcinoma, identified by comparing patients with sustained virological response by univariate analysis using Cox proportional hazard regression method (*n* = 232)

Parameter	F0 + 1 + 2			F3 + 4		
	RR	95%CI	*P* value	RR	95%CI	*P* value
Clinical background factors[1]						
Gender (male)	1.200	0.109-13.252	0.8815	-	-	-
Age (yr)	1.308	0.985-1.737	0.0635	1.123	0.959-1.314	0.1494
BMI	1.121	0.693-1.814	0.6415	0.987	0.549-1.772	0.9644
AST (U/L)	0.996	0.964-1.028	0.7903	1.003	0.971-1.037	0.8540
ALT (U/L)	0.992	0.965-1.020	0.5678	0.996	0.972-1.020	0.7494
γ-GT (U/L)	0.996	0.975-1.018	0.7169	1.001	0.977-1.025	0.9436
Platelet count (× 10^4/μL)	1.004	0.837-1.204	0.9634	1.088	0.855-1.384	0.4936
Genotype type 1	-	-	-	0.285	0.030-2.752	0.2781
AFP (ng/mL)	0.989	0.867-1.129	0.8698	0.995	0.919-1.077	0.9046
Histological findings						
Irregular regeneration[2]						
Dysplastic change	1.920	0.720-5.124	0.1926	1.330	0.543-3.256	0.5322
Oncocytes	-	-	-	1.788	0.510-6.265	0.3639
Map-like distribution	-	-	-	3.082	1.066-8.913	0.0378
Nodularity	-	-	-	-	-	-
Bulging	-	-	-	-	-	-
Atypical hepatocytes	-	-	-	42.055	4.303-411.029	0.0013
Infiltration						
Peri-portal	3.248	0.537-19.655	0.1995	0.477	0.110-2.067	0.3225
Parenchymal	3.028	0.389-23.604	0.2902	0.420	0.092-1.921	0.2634
Portal lymphocyte	2.085	0.217-20.014	0.5241	1.213	0.177-8.333	0.8443
Portal lymphoid reaction	2.362	0.345-16.168	0.3812	3.364	0.484-23.407	0.2202
Fibrosis						
F stage						
Portal sclerosis	0.551	0.078-3.884	0.5500	1.293	0.444-3769	0.6373
Pericellular fibrosis	0.963	0.243-3.822	0.9576	0.534	0.117-2.440	0.4184
Perivenular fibrosis	0.627	0.168-2.344	0.4878	0.429	0.076-2.412	0.3370
Bridging necrosis Y	-	-	-	-	-	-
Bile duct damage	0.678	0.113-4.061	0.6707	1.305	0.374-4.555	0.6766
Steatosis	1.064	0.343-3.306	0.9142	1.660	0.623-4.421	0.3106

We compared the clinical background factors and histological findings at liver biopsy of the patients with sustained virological response in the F0 + 1 + 2 and F3 + 4 groups, data of which are shown. Univariate analysis using the Cox proportional hazard regression method was applied to these parameters to determine the clinical background factors that were associated with the development of hepatocellular carcinoma (HCC). [1]None of the clinical background factors showed any significant associations with HCC. Blank in the table signifies that the bias was applied, but could not be statistically processed; [2]Significant correlations were found for map-like distribution (RR = 3.082, 95%CI: 1.066-8.913, *P* = 0.0378) and atypical hepatocytes (RR = 42.055, 95%CI: 4.303-411.0, *P* = 0.0013) in the F3 + 4 patients. None of the liver histopathological findings showed any significant associations with HCC in the F0 + 1 + 2 patients. Blank values signify that the bias was applied, but could not be statistically processed. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; AFP: α-fetoprotein; BMI: Body mass index; GT: glutamyltransferase.

Table 4 Factors associated with the development of hepatocellular carcinoma, identified by comparing patients with sustained virological response by multivariate analysis using Cox proportional hazard regression method

F3 + 4 parameter	RR	95%CI	*P* value
Map-like distribution	1.687	0.404-7.053	0.4734
Atypical hepatocytes	20.748	1.335-322.530	0.0303

Multivariate analysis using the Cox proportional hazard regression method was applied to the parameters in each of the F stage groups. In the F0 + 1 + 2 group, none of the factors showed any significant correlation with the development of hepatocellular carcinoma (HCC). In the F3 + 4 group, map-like distribution (RR = 1.687, 95%CI: 0.404-7.053, *P* = 0.4734) was not found to have any correlation while the presence of atypical hepatocytes (RR = 20.748, 95%CI: 1.335-322.5, *P* = 0.0303) was shown to be significantly associated with HCC development.

ACKNOWLEDGMENTS

COMMENTS

Background

A few studies have sought to determine the association between the histopathological findings from liver biopsies and the risk of developing hepatocellular carcinoma (HCC). The authors studied 232 patients who underwent liver biopsy, with a sustained virological response (SVR) after interferon (IFN) therapy. They investigated in detail the histopathological findings and analyzed the findings to determine the risk factors.

Research frontiers

It has been reported that progression of liver fibrosis (F stage) constitutes a

risk factor for carcinogenesis. Therefore, the authors sought to identify the liver histopathological findings, other than F stage, that are correlated with the risk of carcinogenesis. If the degree of inflammation and necrosis in the liver were improved following elimination of the virus by IFN therapy, the development of HCC would be attributable, at least in part, to nonviral factors. Then, they investigated the risk factors in detail, including the degree of liver irregular regeneration (IR) and the histopathological findings of liver biopsy specimens, and prospectively examined the findings separately according to the degree of liver fibrosis in SVR patients.

Innovations and breakthroughs

Atypical hepatocytes among IR of hepatocytes may be an important risk factor for HCC development along with progression of liver fibrosis.

Applications

The authors believe that clarification of this finding as a risk factor of carcinogenesis may aid in the early diagnosis of HCC, and it would be meaningful to perform liver biopsy for patients with progression of liver fibrosis. Then, by treating patients for both hepatitis C infection and atypical hepatocytes, they may attain an increase in the survival rate and a lower incidence of HCC.

Terminology

IR of hepatocytes, which occurs as a result of repeated cycles of necrosis and regeneration of the liver parenchyma in chronic hepatitis, is divided into six elements: dysplastic change; anisocytosis characterized by variability of cell size with focal dysplastic change; map-like distribution; distinct populations of hepatocytes with a homogeneous appearance within each population, which are separated from each other by a sharp outline; bulging; expansive proliferation of hepatocytes compressing the surrounding parenchyma. Oncocytes: oncocytic change of hepatocytes. Nodularlity: nodular arrangement of the parenchyma. Atypical hepatocytes: degeneration of hepatocytes.

Peer review

In this study, the authors analyzed the risk factors for HCC associated with type C chronic liver disease. They found that the presence of atypical hepatocytes was significantly correlated with risk of HCC development, and therefore concluded that atypical hepatocytes among the histopathological findings of type C liver disease may be an important risk factor for HCC development along with progression of liver fibrosis.

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