Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 21; 13(39): 5180-5187
World Journal of Gastroenterology ISSN 1007-9327

LIVER CANCER

Hepatic steatosis as a possible risk factor for the development of hepatocellular carcinoma after eradication of hepatitis C virus with antiviral therapy in patients with chronic hepatitis C

Atsushi Tanaka, Satoko Uegaki, Hiroko Kurihara, Kiyoshi Aida, Masaki Mikami, Ikuo Nagashima, Junji Shiga, Hajime Takikawa

Atsushi Tanaka, Satoko Uegaki, Hiroko Kurihara, Masaki Mikami, Hajime Takikawa, Department of Medicine, Teikyo University School of Medicine, Kaga, Itabashi-ku, Tokyo 173-8605, Japan
Kiyoshi Aida, Junji Shiga, Department of Pathology, Teikyo University School of Medicine, Kaga, Itabashi-ku, Tokyo 173-8605, Japan
Ikuo Nagashima, Department of Surgery, Teikyo University School of Medicine, Kaga, Itabashi-ku, Tokyo 173-8605, Japan
Correspondence to: Atsushi Tanaka, MD, Department of Medicine, Teikyo University School of Medicine, 2-11-1, Kaga, Itabashi-ku, Tokyo 173-8605, Japan. a-tanaka@med.teikyo-u.ac.jp
Telephone: +81-3-39641211 Fax: +81-3-53751308
Received: April 18, 2007 Revised: June 28, 2007

Key words: Hepatitis C virus; Chronic hepatitis C; Hepatocellular carcinoma; Hepatic steatosis; Hepatic fibrosis; Interferon therapy; Sustained viral response

Tanaka A, Uegaki S, Kurihara H, Aida K, Mikami M, Nagashima I, Shiga J, Takikawa H. Hepatic steatosis as a possible risk factor for the development of hepatocellular carcinoma after eradication of hepatitis C virus with antiviral therapy in patients with chronic hepatitis C. *World J Gastroenterol* 2007; 13(39): 5180-5187

http://www.wjgnet.com/1007-9327/13/5180.asp

Abstract

AIM: To elucidate risk factors contributing to the development of hepatocellular carcinoma (HCC) among patients with sustained viral response (SVR) after interferon (IFN) treatment and to examine whether HCV-RNA still remained in the liver of SVR patients who developed HCC.

METHODS: Two-hundred and sixty-six patients, who achieved SVR, were enrolled in this study. We retrospectively reviewed clinical, viral and histological features of the patients, and examined whether the development of HCC depends on several clinical variables using Kaplan-Meier Method. RT-PCR was used to seek HCV-RNA in 3 out of 7 patients in whom liver tissue was available for molecular analysis.

RESULTS: Among the enrolled 266 patients with SVR, HCC developed in 7 patients (7/266; 2.6%). We failed to detect HCV-RNA both in cancer and non-cancerous liver tissue in all three patients. The cumulative incidence for HCC was significantly different depending on hepatic fibrosis (F3-4) (P = 0.0028), hepatic steatosis (Grade 2-3) (P = 0.0002) and age ($\geqslant$ 55) (P = 0.021) at the pre-interferon treatment.

CONCLUSION: The current study demonstrated that age, hepatic fibrosis, and hepatic steatosis at pre-interferon treatment might be risk factors for developing HCC after SVR.

INTRODUCTION

Continuous eradication of hepatitis C virus (HCV) with interferon (IFN) therapy significantly inhibits development of hepatocellular carcinoma (HCC) in patients with chronic hepatitis C[1-3]. However, HCC sporadically developed even after achievement of sustained viral response (SVR) with IFN therapy, especially reported from Japan[4-14], and therefore it is of clinical importance to identify patients who are at high risk for HCC after achievement of SVR.

Several investigators have made efforts to elucidate risk factors for occurrence of HCC in patients with SVR[6,15-18], and so far several factors including age[6,15-18], sex[6,15], alcohol consumption[16,17], staging of fibrosis[15-17], platelet counts[18], and AST levels[18] at baseline were regarded as candidate risks. However, these results seem to be controversial, and there may be other crucial contributing factors. In addition, reappearance of HCV in serum after achievement of SVR has also been reported[19-22]. In some cases, HCV-RNA is still present in liver of patients without detectable HCV-RNA in serum[19-22]. Therefore, the remaining HCV-RNA in the liver may contribute to the occurrence of HCC in patients with SVR[23]. Nevertheless, the studies[6,15-18] describing risk factors for the occurrence of HCC lack any evaluation for the presence of HCV-RNA in the liver.

Furthermore, chronic HCV infection frequently results in hepatic steatosis[24]. It has been reported that HCV genotype 3 infection is likely to cause steatosis possibly due to direct effect of HCV, whereas steatosis in patients infected with HCV genotype 1 seems to be associated

with co-existing risk factors including an increased body mass index (BMI) or presence of insulin resistance, thus resembling to those non-alcoholic steatohepatitis (NASH)[25-29]. Apparently hepatic steatosis is not altered in patients infected with HCV genotype 1, even after continuous eradication of HCV with antiviral therapy[30]. In addition, obesity-related cryptogenic cirrhosis has been recently paid attention as an alternative etiology for HCC[31,32]. Taken together, it may be possible that hepatic steatosis, still present after achievement of eradication of HCV genotype 1, results in progression of fibrosis as well as occurrence of HCC. The investigators describing risk factors for HCC in patients with SVR[6,15-18] have not mentioned whether hepatic steatosis is observed in pre-treatment liver histology.

Therefore, in the current study, we aimed to elucidate contributing factors for occurrence of HCC in patients in whom serum HCV-RNA has been eradicated with IFN therapy. First, we examined whether HCV-RNA still remained in the liver at the development of HCC using sensitive reverse-transcriptase polymerase chain reaction (RT-PCR). In addition, we evaluated clinical features and histopathological fibrosis and steatosis of the patients at the pre-IFN treatment, and examined whether the occurrence of HCC depends on these factors using Kaplan-Meier model.

MATERIALS AND METHODS

Patients

This single-center study was conducted at the Department of Medicine, Teikyo University School of Medicine. Since the introduction of IFN therapy for patients with chronic hepatitis C in 1986, 1101 patients were treated with IFN alone or IFN and ribavirin combination therapy. Among them, 266 patients, who achieved SVR, defined as absence of serum HCV-RNA at 6 mo after termination of IFN therapy, and did not fulfill the exclusion criteria described below, were enrolled. We verified that anti-HCV antibody as well as HCV-RNA was detected in sera before IFN treatment in all enrolled patients. Serum anti-HCV antibody was examined using third-generation antibody to HCV (Abbott Japan, Tokyo, Japan). Serum HCV-RNA was sought with Amplicor HCV v2.0 (Nippon Roche, Tokyo, Japan), employing RT-PCR. Also, liver biopsy was performed in all cases as long as 3 mo before beginning of IFN therapy for assuring the presence of chronic hepatitis. Exclusion criteria for this study were hepatitis B virus (HBV) infection determined by seropositivity for HBsAg and/or HBcAb, autoimmune hepatitis, alcoholic abuse (daily alcohol consumption > 60 g), and presence of HCC detected by abdominal ultrasound (US) and/or computed tomography (CT).

Follow-up and diagnosis of hepatocellular carcinoma

After termination of IFN therapy, patients were regularly checked up at the out-patient clinic at every 2-3 mo. The average period of follow-up for the enrolled patients in this study was 9.9 ± 4.1 years. Blood chemistries as well as tumor markers were examined. Additionally, imaging studies, abdominal US and/or CT, were performed 1-2 times per year. The diagnosis of HCC was made if both abdominal US and CT demonstrated the presence of HCC, and was further confirmed by histopathological studies after surgical resection. In cases without resection, abdominal angiography was used to verify the diagnosis of HCC.

Variables

We selected several pre-IFN treatment valuables of patients to assess risk factors for development of HCC, including the age at pre-IFN treatment and sex of the patient, the stage of liver fibrosis and the grade of hepatic steatosis, HCV genotype and serum ALT level. The stage of liver fibrosis was determined by two independent pathologists according to the classification of the Metavir group[33] as follows: F0 (no fibrosis); F1 (portal fibrosis); F2 (few bridges); F3 (many bridges); and F4 (cirrhosis). Hepatic steatosis was also graded by two independent pathologists according to Brunt *et al*[34]. In short, steatosis observed in up to 33%, 33%-66% and more than 66% of the liver histology was determined as grade 1, 2 and 3, respectively. Hepatic steatosis, if not observed, was graded as grade 0. When two pathologists estimated the classification of fibrosis and the grading of steatosis differently, the mean value of the two was applied for statistical analysis. Genotyping of HCV-RNA was performed with HCV coregenotyping (Nippon Roche).

Detection of HCV-RNA in liver tissue

HCV-RNA in liver tissue was detected by RT-PCR. Formalin-embedded liver biopsy specimens obtained prior to IFN treatment and snap-frozen liver tissue taken at the development of HCC were used as starting materials. Total RNA was extracted using Isogen (Nippon gene, Tokyo, Japan) and was converted into cDNA using Superscript™ Ⅱ RNase H⁻ Reverse Transcriptase (Invitrogen, Carlsbad, CA). The primer specific to HCV-RNA 5' UTR region (HC1R: ACTCGCAAGCACCCTATCA, nt 293-312) was used for cDNA synthesis. Thereafter HCV-RNA was sought using "semi-nested" PCR consisting of 1st, 2nd and 3rd amplification, using one 5' forward primer (HC1F: GAGCCATAGTGGTCTGCGGA, nt 135-154) and three sets of reverse primers (HC1R, HC2R: ACT CGGCTAGCAGTCTTGCG, nt 240-259; and 2H-AS: GTTTATCCAAGAAAGGACCC, nt 188-207). The 1st PCR consisted of initial denaturing at 95℃ for 5 min, followed by 50 amplification cycles at 95℃ for 15 s for denaturing and at 62℃ for 1 min for primer annealing and extension, and a final extension at 72℃ for 7 min. The 2nd PCR was done for 35 cycles by the identical program, but annealing and extension at 63℃. The 3rd PCR consisted of initial denaturing at 95℃ for 5 min, followed by 30 amplification cycles at 95℃ for 15 s, at 58℃ for 30 s for primer annealing and at 72℃ for 1 min for extension, and a final extension at 72℃ for 7 min. The PCR products of 178, 125 and 73 bp after 1st, 2nd and 3rd PCR, respectively, were electrophoresed on 15 g/L agarose gel, and visualized by ethidium bromide staining. In addition, to ensure the quality of extracted RNA, we also amplified β-actin as positive controls using the identical liver-derived total

Table 1 Viral, clinical and histopathological profiles of patients with SVR who developed HCC

	Age/sex	HCV genotype	Pre-IFN treatment				Occurrence of HCC			Duration until HCC (yr)
			ALT (IU/L)	Fibrosis	Steatosis	BMI	Fibrosis	Steatosis	BMI	
Case 1	67/M	1b	138	F3	Grade 2	28.4	F2	Grade 1	27.4	5.4
Case 2	58/M	2a	39	F1	Grade 1	23.5	F2	Grade 0	22.5	9.5
Case 3	68/M	n.t.	111	F4	Grade 2	24.2	F2	Grade 2	25.0	9.6
Case 4	53/M	1b	82	F2	Grade 1	19.6	NT	NT	20.3	8.4
Case 5	58/M	1b	21	F3	Grade 1	23.5	NT	NT	24.8	3.3
Case 6	65/F	2a	145	F3	Grade 1	25.6	F2	Grade 1	26.7	2.9
Case 7	54/M	1b	85	F2	Grade 2	26.1	F2	Grade 1	22.4	4.9

NT: Not tested; BMI: Body mass index. In cases 4 and 5, the liver tissues with the occurrence of HCC were not obtained because HCC was not treated with surgical operation.

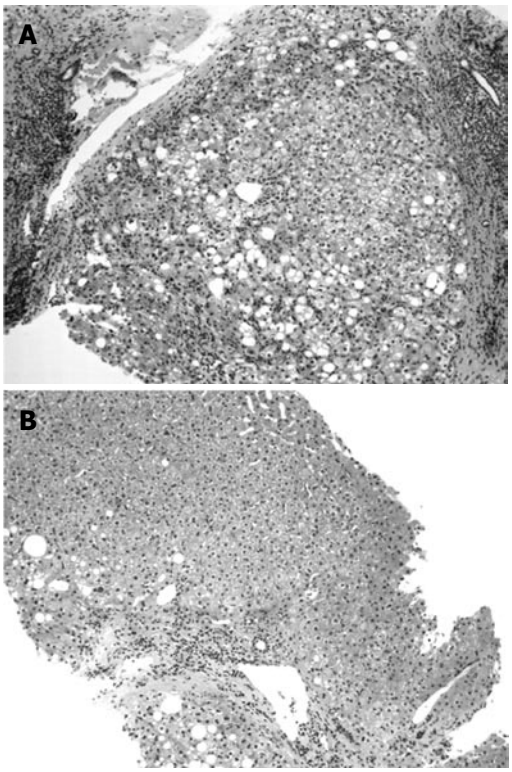

Figure 1 Liver histology of case 1 (HE staining). **A**: Pre-IFN treatment liver histology showing F3 fibrosis grade 2 steatosis; **B**: Liver histology at the occurrence of HCC showing F2 fibrosis and grade 1 steatosis.

RNA. Random primer was used for synthesis of cDNA as template for amplification of β-actin.

Statistical analysis

Statistical analysis was performed using SAS software version 9.1 (SAS Institute, Cary, NC). The kappa value was calculated to evaluate the degree of agreement in two pathologists for histological evaluation. For determining risk factors contributing the occurrence of HCC, continuous variables such as age and pre-treatment ALT value were classified into two categories: age $\geqslant$ 55 and age $<$ 55; ALT $>$ 80 IU/L and ALT $\leqslant$ 80 IU/L. Cumulative incidence for development of HCC was calculated using Kaplan-Meier method, and the differences between groups were analyzed using the log-rank test. *P* value less than 0.05. was considered statistically significant.

RESULTS

Patients with SVR who developed HCC

Of 266 patients, HCC was detected in 7 patients (6 males and 1 female) during follow-up for the patients with SVR after termination of IFN therapy. Clinical and histopathological profiles of these patients are mentioned in Table 1. The age and mean ALT value at the beginning of IFN therapy was 60.4 ± 6.2 (range, 53-68) years, and 89 ± 47 (range, 21-145) IU, respectively. The duration between IFN therapy and development of HCC was 6.3 ± 2.9 (range, 2.9-9.6) years. Undetectable serum HCV-RNA and normal ALT had been continuously maintained since IFN therapy in all patients. All patients denied excess consumption of alcoholic beverage (> 20 g/d), and were seronegative for HBsAg as well as HBcAb. The staging of liver fibrosis on pre-IFN liver biopsy was F1 in one patient, F2 in two, F3 in three and F4 in one. Hepatic steatosis was observed in all patients, grade 1 in four patients and grade 2 in three.

The comparison of liver histological findings at baseline and at the development of HCC is shown in Table 1. Overall, the grading of fibrosis was improved in cases 1, 3 and 6, unchanged in case 7, and worsened in case 2. Steatosis was decreased in cases 1, 2 and 7, and unchanged in cases 3 and 6. In cases 4 and 5, we failed to obtain liver tissue at the development of HCC since these patients were treated by transcatheter arterial embolization, instead of surgical resection of the tumor. Liver histology of case 1 before IFN therapy and at the occurrence of HCC as a representative example is shown in Figure 1.

Failure to detect HCV-RNA in HCC

In 3 patients (cases 1, 2 and 4), we tried to detect HCV-RNA in liver tissue before IFN treatment as well as at the development of HCC to elucidate whether HCV-RNA, which could remain in the liver tissue even long after successful IFN treatment[22], might be involved in hepatocellular carcinogenesis. The tumors were surgically resected in cases 1 and 2, and the liver specimens were obtained from both the cancerous as well as non-cancerous lesion and snap-frozen at -80℃ until use. In case 4, as described above, the HCC was treated by transcatheter arterial embolization, and thus the liver specimens at occurrence of HCC were not available. Instead, biopsied specimen from the liver obtained after

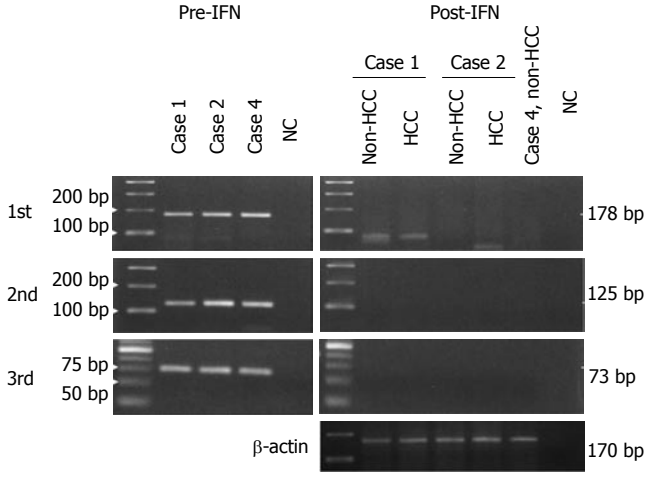

Figure 2 Amplification of HCV-RNA in liver tissues in cases 1, 2 and 4. HCV was detected in all liver tissues at the pre-IFN therapy. NC: Without template cDNA, gave no distinct band. By contrast, HCV was demonstrated in neither HCC nor non-HCC tissue in cases 1 and 2 even after 3 cycles of RT-PCR. In case 4, in whom liver tissue was obtained after IFN therapy with percutaneous biopsy, HCV was not found either. β-actin was well amplified in these post-IFN specimens, demonstrating that total RNA had not been degraded. NC: Negative control.

IFN therapy was used. HCV-RNA was successfully amplified in all specimens before IFN treatment. However, we failed to detect HCV-RNA in any sample, irrespective of cancerous and non-cancerous tissue (Figure 2). β-actin was successfully amplified in all liver tissue after IFN therapy and at HCC development (Figure 2). Therefore, we concluded that failure to detect HCV-RNA in liver tissue at development of HCC was not due to degradation of RNA, but resulted from disappearance of HCV-RNA in the liver with successful IFN treatment.

Cumulative incidence of HCC during follow-up

We demonstrated viral, clinical and histopathological profiles in all enrolled patients, the patients with and without HCC (Table 2). The cumulative incidence of HCC was analyzed for 4 variables which had been reported as risk factors before (Figure 3A-D), and pre-treatment hepatic steatosis (Figure 3E). As for the histopathological evaluation, there was an excellent agreement in the histopathological evaluation by two independent pathologists, since the kappa value was 0.70 in fibrosis and 0.78 in steatosis. The age of patients with/without HCC at baseline was 60.4 ± 6.2 and 44.8 ± 13.3 (Table 2), respectively and the patients with age ⩾ 55 were at significantly higher risk for the development of HCC (P = 0.021, Figure 3A). The staging of pre-treatment hepatic fibrosis was available in 238 of 266 patients. The number of patients with F1, F2, F3 and F4 was 1, 2, 3, 1 in patients with HCC and 102, 97, 25, 7 in patients without HCC, respectively (Table 2). Log-rank test analysis revealed that advanced hepatic fibrosis (F3-4) was a risk factor for HCC as well (P = 0.0028, Figure 3C). Pre-IFN hepatic steatosis was evaluated in 231 of 266 patients, and the number of patients in grade 0, 1, 2 and 3 was 116, 95, 19 and 1, respectively. It is notable that no patient in grade 0 (no steatosis) at the IFN pre-treatment developed HCC, whereas 3 of 19 patients with grade 2 steatosis developed HCC in the follow-up (4.9, 5.4 and 9.6 years after IFN therapy, respectively) (Table 2). Indeed, log-rank test analysis demonstrated that there was a significant difference in the grade of steatosis for cumulative incidence of HCC (P = 0.0002, Figure 3E). By contrast, we failed to demonstrate that sex and pre-treatment ALT value were risk factors for HCC (P = 0.389 and 0.251, respectively, Figure 3C and D).

DISCUSSION

Among 266 cases, who achieved SVR with IFN therapy, 7 patients developed HCC in the follow-up period. Statistical analysis using log-rank test demonstrated that the cumulative incidence of development of HCC after SVR was significantly different depending on age and hepatic fibrosis, as previously suggested, and also on hepatic steatosis in these patients.

In the previous investigations, age[6,15-18], sex[6,15], alcohol consumption[16,17], staging of fibrosis[15-17], platelet counts[18], and AST levels[18] at baseline were regarded as risk factors for occurrence of HCC in patients with SVR. In the current study, we selected age, sex, ALT and staging of fibrosis as candidate contributing variables. Excess consumption of alcohol beverage was not noted in any patient with HCC and therefore was very unlikely to be a risk factor in the enrolled population. Since it is assumed that platelet counts are equivalent to staging of fibrosis and that serum ALT levels are more specific to hepatocellular injury, we adapted fibrosis and ALT values, instead of platelet counts and AST levels.

The exact determination of stage of hepatic fibrosis as well as the grade of hepatic steatosis at pre-IFN treatment is a vital premise in the current study. In this regard, grading of fibrosis and grading of steatosis were performed by two independent pathologists. The kappa values were 0.70 in fibrosis and 0.78 in steatosis, and there was a good agreement in the decision of the two. The results appeared comparable to previous reports[18]. For instance, the proportion of the patients graded in F1, F2, F3 and F4 was 43%, 42%, 12% and 3% in the current study, and 41%, 30%, 17% and 10% in the report by Ikeda *et al*[18] who included 1056 patients with SVR. In addition, hepatic steatosis in our study was observed in 115 of 231 (50%) patients evaluated, and it is generally accepted that steatosis is present in 40%-60% of the patients with chronic hepatitis C[24]. Therefore, it is conceivable to conclude that hepatic fibrosis as well as steatosis were adequately assessed in this study.

In the current study, the incidence of the patients who developed HCC after SVR was only 2.6% (7/266). Therefore, in the current study, we performed statistical analysis with only Kaplan-Meier method, and thus the effect of confounding factors among each valuable could not be excluded. In this regard, we should be reluctant to conclude that age, hepatic fibrosis and hepatic steatosis, demonstrated in this study as statistically significant, would be the only determinants of risk for developing HCC. Nevertheless, hepatic steatosis, which has never been investigated as a risk factor for developing HCC after SVR, is identified as a statistically significant factor, and

Table 2 Pre-IFN viral, clinical and histopathological profiles of all patients with SVR

	Age (mean ± SD)	Sex (M/F)	ALT (IU) (mean ± SD)	Genotype (1/2/NT)	Hepatic fibrosis (F1/F2/F3/F4/NT)	Hepatic steatosis (Grade 0/1/2/3/NT)
All (n = 266)	46.3 ± 14.3	184/82	112 ± 99	102/142/20	103/99/28/8/28	116/95/19/1/35
HCC (n = 7)	60.4 ± 6.2	6/1	89 ± 47	4/2/1	1/2/3/1/0	0/4/3/0/0
No HCC (n = 259)	44.8 ± 13.3	178/81	113 ± 100	98/140/19	102/97/25/7/28	116/91/16/1/35

NT: Not tested.

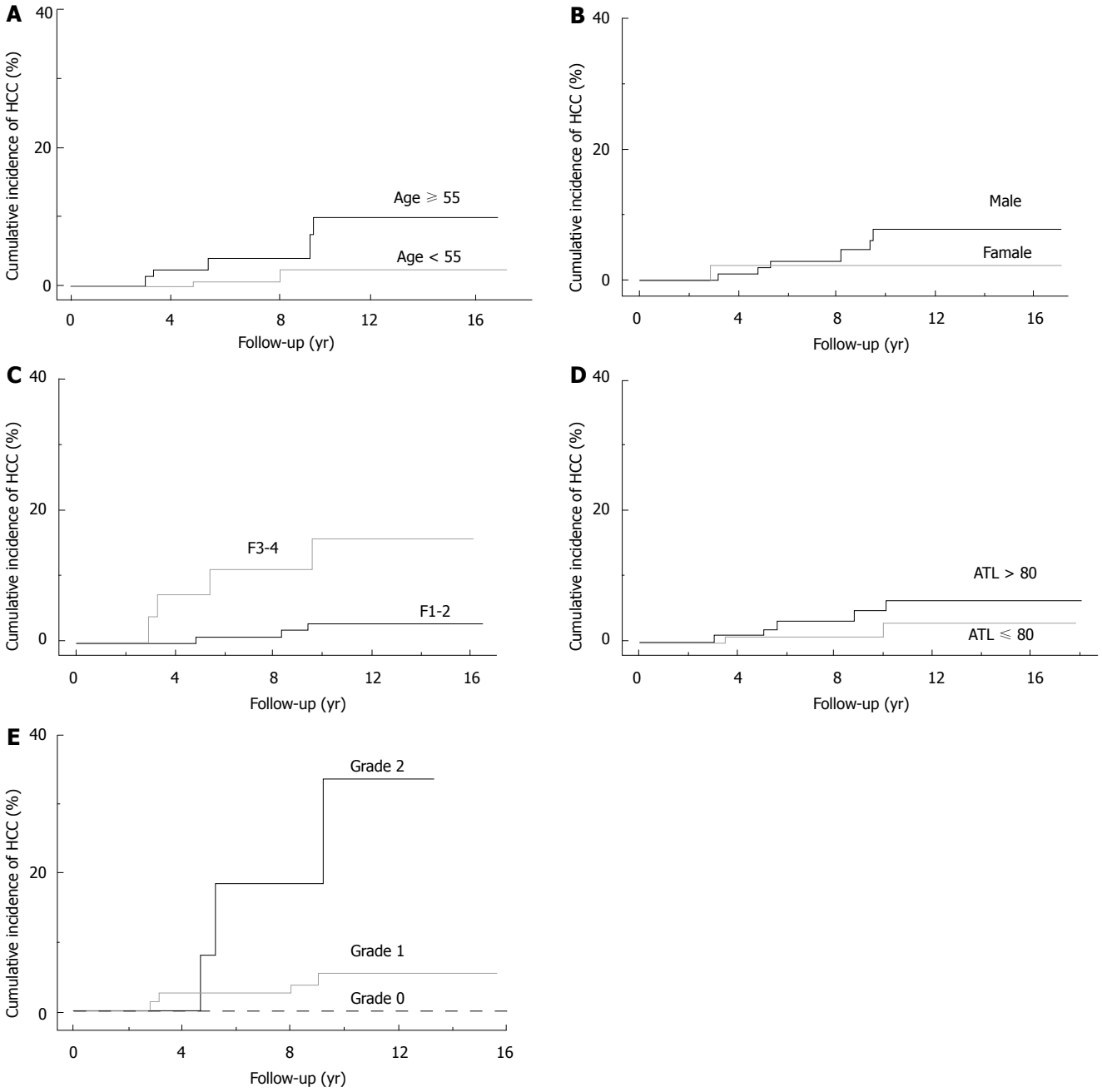

Figure 3 Cumulative incidence of HCC in SVR patients using the log-rank test. **A**: Patients with age ⩾ 55 (black line) and < 55 (gray line) years (P = 0.021); **B**: Male (black line) and female (gray line) patients (P = 0.389); **C**: patients with grading of fibrosis, F3-4 (black line) and F1-2 (gray line) (P = 0.0028); **D**: Patients with pre-treatment ALT levels, ALT > 80 IU (black line) and ⩽ 80 IU (gray line) (P = 0.251); **E**: Patients with the grading of hepatic steatosis, grade 2 (black line), grade 1 (gray line), and grade 0 (gray dotted line) (P = 0.0002 between grade 2 and grade 1 or grade 0).

we believe that it is worth evaluating its potential role for developing HCC after eradication of HCV.

Hepatic steatosis is known to be decreased after eradication of HCV in HCV genotype 3 infection. However, HCV genotype 3 is very rare in Japan, and indeed we found no patient infected with genotype 3 among the 266 subjects enrolled in the current study. By contrast, it has been reported that hepatic steatosis with HCV genotype 1

infection is not altered after successful antiviral treatment[30]. In the current study, among 6 patients who developed HCC after SVR and in whom HCV genotype was examined, 4 and 2 were infected with genotype 1 and 2, respectively (Table 1). Disappearance of hepatic steatosis (grade 0) after successful IFN therapy was noted only in 1 patient (case 2), infected with HCV genotype 2a; steatosis still remained in 4 of 5 patients whose histologies on the occurrence of HCC were investigated (Table 1). Thus, hepatic steatosis at baseline continuously remained after eradication of HCV, and might play a role in development of HCC, along with other factors, such as age and fibrosis in the liver.

It has been repeatedly reported that NASH caused by hepatic steatosis eventually could result in development of HCC[31,32,35-39], and indeed NASH is regarded as an alternative etiology of HCC worldwide[36,40,41]. It is believed that cryptogenic cirrhosis, occasionally producing HCC, may be a late complication of NASH, even though steatosis is not observed in the end-stage cirrhotic liver[31,32]. In the current study, however, liver histology on the onset of HCC was not coincident with cryptogenic cirrhosis. Rather, the staging of fibrosis was F2 in 5 of 7 patients who developed HCC, relatively at early stage. Moreover, although hepatic steatosis was noted at the pre-IFN treatment, histological hallmarks of NASH, such as perisinusoidal/pericellular fibrosis or infiltration of polymorphonuclear cells[42,43], were not observed in our patients. Therefore, hepatic steatosis in these patients should be regarded as non-alcoholic fatty liver diseases (NAFLD), instead of NASH. Taken together, the role of hepatic steatosis in the hepatocarcinogenesis after SVR seems to be different from those in NASH liver. Rather, the results in the current study suggest the possibility that steatosis and HCV infection, even if eradicated, would cooperatively operate for development of HCC.

We should be cautious, however, in concluding that hepatic steatosis would be a risk factor for developing HCC after SVR. First of all, multivariate analysis was not performed in this study and therefore the effect of confounding factors could not be excluded, as described above. Second, it is still controversial whether hepatic steatosis facilitates hepatocarcinogenesis in untreated patients with infection of HCV[44,45], and prospective well-controlled studies are required to conclude the association between hepatic steatosis and development of HCC. Third, it is also notable that HCC developed within 10 years after successful eradication of HCV in the presented cases, even though almost half of the enrolled 266 patients with SVR had been followed up more than 10 years. Since hepatic steatosis would remain in a similar manner after eradication of HCV, HCC might develop in patients after more than 10 years as well. Finally, very low amount of HCV, even undetectable by RT-PCR, had still remained in the liver after SVR and might play a crucial role in hepatocarcinogenesis. Radkowski *et al*[22] recently demonstrated that HCV-RNA was detected in 3 of 11 patients with SVR after treatment, even though previous reports describing the occurrence of HCC in patients with SVR have repeatedly shown the absence of HCV-RNA in the tumor and non-tumor liver tissue[8,9,12].

In conclusion, the current study demonstrates that development of HCC in patients after SVR depends on age, hepatic fibrosis, and hepatic steatosis at pre-IFN treatment. As discussed, the remote effect of HCV infection could be more important for hepatocarcinogenesis, and a large-scale, multi-center cooperative study is required to conclude whether hepatic steatosis at baseline is a contributing factor for development of HCC. Nevertheless, it should be kept in mind that patients with hepatic steatosis at pre-IFN treatment may be at a high risk for developing HCC, and therefore should be closely monitored after SVR.

COMMENTS

Background

Continuous eradication of hepatitis C virus (HCV), i.e., sustained viral response (SVR) with interferon (IFN) therapy greatly reduces the risk of developing hepatocellular carcinoma (HCC). However, HCC was sporadically found even in patients with SVR, and it is of clinical importance to elucidate which patients with SVR are at higher risk for HCC.

Research frontiers

In the current study, we retrospectively examined 266 patients with SVR and detected 7 patients among them (2.6%) who developed HCC after SVR. RT-PCR failed to detect HCV-RNA in the liver. The cumulative incidence for HCC was significantly different depending on hepatic fibrosis (P = 0.0028), hepatic steatosis (P = 0.0002) and higher age (P = 0.021) at the pre-interferon treatment.

Innovations and breakthroughs

Although age and fibrosis were previously reported as risk factors, hepatic steatosis was firstly demonstrated as a possible risk in the current study.

Applications

Further large-scale study is warranted to confirm the contribution of hepatic steatosis for developing HCC after SVR. For the moment, patients with high hepatic steatosis should be closely monitored for HCC, even after SVR with successful antiviral treatment.

Peer review

This is a very interesting study. There are few papers with such histological and follow up studies in SVR and the article is well written.

REFERENCES

1 **Yoshida H**, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, Inoue O, Yano M, Tanaka M, Fujiyama S, Nishiguchi S, Kuroki T, Imazeki F, Yokosuka O, Kinoyama S, Yamada G, Omata M. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. *Ann Intern Med* 1999; **131**: 174-181

2 **Cammà C**, Giunta M, Andreone P, Craxì A. Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. *J Hepatol* 2001; **34**: 593-602

3 **Shiratori Y**, Ito Y, Yokosuka O, Imazeki F, Nakata R, Tanaka N, Arakawa Y, Hashimoto E, Hirota K, Yoshida H, Ohashi Y, Omata M. Antiviral therapy for cirrhotic hepatitis C: association with reduced hepatocellular carcinoma development and improved survival. *Ann Intern Med* 2005; **142**: 105-114

4 **Hirashima N**, Mizokami M, Orito E, Koide T, Itazu I, Kumada K, Sakakibara K, Kano H, Lau JY. Case report: development of hepatocellular carcinoma in a patient with chronic hepatitis C infection after a complete and sustained response to interferon-alpha. *J Gastroenterol Hepatol* 1996; **11**: 955-958

5 **Tamori A**, Kuroki T, Nishiguchi S, Morimoto H, Morimoto M, Hirohashi K, Kinoshita AH, Kobayashi K. Case of small hepatocellular carcinoma in the caudate lobe detected after interferon caused disappearance of hepatitis C virus. *Hepatogastroenterology* 1996; **43**: 1079-1083

6 **Kasahara A**, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, Iijima A, Urushihara A, Kiyosawa K, Okuda M, Hino K, Okita K. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. *Hepatology* 1998; **27**: 1394-1402

7 **Miyano S**, Togashi H, Shinzawa H, Sugahara K, Matsuo T, Takeda Y, Saito K, Saito T, Ishiyama S, Kaneko M, Takahashi T. Case report: Occurrence of hepatocellular carcinoma 4.5 years after successful treatment with virus clearance for chronic hepatitis C. *J Gastroenterol Hepatol* 1999; **14**: 928-930

8 **Yamaguchi K**, Omagari K, Kinoshita H, Yoshioka S, Furusu H, Takeshima F, Nanashima A, Yamaguchi H, Kohno S. Development of hepatocellular carcinoma in a patient with chronic hepatitis C after 6 years of a sustained and complete response to IFN-alpha. *J Clin Gastroenterol* 1999; **29**: 207-209

9 **Inoue M**, Ohhira M, Ohtake T, Matsumoto A, Kawashima T, Fujimoto Y, Hasebe C, Ono M, Kohgo Y. Hepatocellular carcinoma developed in a patient with chronic hepatitis C after the disappearance of hepatitis C virus due to interferon therapy. *Hepatogastroenterology* 1999; **46**: 2554-2560

10 **Yamada M**, Ichikawa M, Matsubara A, Ishiguro Y, Yamada M, Yokoi S. Development of small hepatocellular carcinoma 80 months after clearance of hepatitis C virus with interferon therapy. *Eur J Gastroenterol Hepatol* 2000; **12**: 1029-1032

11 **Toyoda H**, Kumada T, Honda T, Hayashi K, Katano Y, Nakano I, Hayakawa T, Fukuda Y. Analysis of hepatocellular carcinoma tumor growth detected in sustained responders to interferon in patients with chronic hepatitis C. *J Gastroenterol Hepatol* 2001; **16**: 1131-1137

12 **Yamaura T**, Matsumoto A, Rokuhara A, Ichijo T, Tanaka E, Hanazaki K, Kajikawa S, Kiyosawa K. Development of small hepatocellular carcinoma in a patient with chronic hepatitis C after 77 months of a sustained and complete response to interferon therapy. *J Gastroenterol Hepatol* 2002; **17**: 1229-1235

13 **Tomimatsu M**, Endo H, Kitazawa M, Iga D, Fujimoto T, Ohkawa S, Kajiyama H, Katagiri S, Harada N, Yamamoto M, Takasaki K. Type C chronic hepatitis with the discovery of a small hepatocellular carcinoma 7 years after successful interferon therapy. *J Gastroenterol* 2003; **38**: 395-398

14 **Enokimura N**, Shiraki K, Kawakita T, Saitou Y, Inoue H, Okano H, Yamamoto N, Deguchi M, Sakai T, Ohmori S, Fujikawa K, Murata K, Niki Y, Nakano T. Hepatocellular carcinoma development in sustained viral responders to interferon therapy in patients with chronic hepatitis C. *Anticancer Res* 2003; **23**: 593-596

15 **Makiyama A**, Itoh Y, Kasahara A, Imai Y, Kawata S, Yoshioka K, Tsubouchi H, Kiyosawa K, Kakumu S, Okita K, Hayashi N, Okanoue T. Characteristics of patients with chronic hepatitis C who develop hepatocellular carcinoma after a sustained response to interferon therapy. *Cancer* 2004; **101**: 1616-1622

16 I**wasaki Y**, Takaguchi K, Ikeda H, Makino Y, Araki Y, Ando M, Kobashi H, Kobatake T, Tanaka R, Tomita M, Senoh T, Kawaguchi M, Shimoe T, Manabe K, Kita K, Shimamura J, Sakaguchi K, Shiratori Y. Risk factors for hepatocellular carcinoma in Hepatitis C patients with sustained virologic response to interferon therapy. *Liver Int* 2004; **24**: 603-610

17 **Tokita H**, Fukui H, Tanaka A, Kamitsukasa H, Yagura M, Harada H, Okamoto H. Risk factors for the development of hepatocellular carcinoma among patients with chronic hepatitis C who achieved a sustained virological response to interferon therapy. *J Gastroenterol Hepatol* 2005; **20**: 752-758

18 **Ikeda M**, Fujiyama S, Tanaka M, Sata M, Ide T, Yatsuhashi H, Watanabe H. Risk factors for development of hepatocellular carcinoma in patients with chronic hepatitis C after sustained response to interferon. *J Gastroenterol* 2005; **40**: 148-156

19 **Reichard O**, Glaumann H, Frydén A, Norkrans G, Wejstål R, Weiland O. Long-term follow-up of chronic hepatitis C patients with sustained virological response to alpha-interferon. *J Hepatol* 1999; **30**: 783-787

20 **McHutchison JG**, Poynard T, Esteban-Mur R, Davis GL, Goodman ZD, Harvey J, Ling MH, Garaud JJ, Albrecht JK, Patel K, Dienstag JL, Morgan T. Hepatic HCV RNA before and after treatment with interferon alone or combined with ribavirin. *Hepatology* 2002; **35**: 688-693

21 **Veldt BJ**, Saracco G, Boyer N, Cammà C, Bellobuono A, Hopf U, Castillo I, Weiland O, Nevens F, Hansen BE, Schalm SW. Long term clinical outcome of chronic hepatitis C patients with sustained virological response to interferon monotherapy. *Gut* 2004; **53**: 1504-1508

22 **Radkowski M**, Gallegos-Orozco JF, Jablonska J, Colby TV, Walewska-Zielecka B, Kubicka J, Wilkinson J, Adair D, Rakela J, Laskus T. Persistence of hepatitis C virus in patients successfully treated for chronic hepatitis C. *Hepatology* 2005; **41**: 106-114

23 **Basso M**, Torre F, Picciotto A. Characteristics of patients with chronic hepatitis C who develop hepatocellular carcinoma after a sustained response to interferon therapy. *Cancer* 2005; **103**: 2654-2655; author reply 2655

24 **Ramesh S**, Sanyal AJ. Hepatitis C and nonalcoholic fatty liver disease. *Semin Liver Dis* 2004; **24**: 399-413

25 **Adinolfi LE**, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 2001; **33**: 1358-1364

26 **Monto A**, Alonzo J, Watson JJ, Grunfeld C, Wright TL. Steatosis in chronic hepatitis C: relative contributions of obesity, diabetes mellitus, and alcohol. *Hepatology* 2002; **36**: 729-736

27 **Sharma P**, Balan V, Hernandez J, Rosati M, Williams J, Rodriguez-Luna H, Schwartz J, Harrison E, Anderson M, Byrne T, Vargas HE, Douglas DD, Rakela J. Hepatic steatosis in hepatitis C virus genotype 3 infection: does it correlate with body mass index, fibrosis, and HCV risk factors? *Dig Dis Sci* 2004; **49**: 25-29

28 **Rubbia-Brandt L**, Fabris P, Paganin S, Leandro G, Male PJ, Giostra E, Carlotto A, Bozzola L, Smedile A, Negro F. Steatosis affects chronic hepatitis C progression in a genotype specific way. *Gut* 2004; **53**: 406-412

29 **Castéra L**, Hézode C, Roudot-Thoraval F, Lonjon I, Zafrani ES, Pawlotsky JM, Dhumeaux D. Effect of antiviral treatment on evolution of liver steatosis in patients with chronic hepatitis C: indirect evidence of a role of hepatitis C virus genotype 3 in steatosis. *Gut* 2004; **53**: 420-424

30 **Kumar D**, Farrell GC, Fung C, George J. Hepatitis C virus genotype 3 is cytopathic to hepatocytes: Reversal of hepatic steatosis after sustained therapeutic response. *Hepatology* 2002; **36**: 1266-1272

31 **Bugianesi E**, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002; **123**: 134-140

32 **Ratziu V**, Bonyhay L, Di Martino V, Charlotte F, Cavallaro L, Sayegh-Tainturier MH, Giral P, Grimaldi A, Opolon P, Poynard T. Survival, liver failure, and hepatocellular carcinoma in obesity-related cryptogenic cirrhosis. *Hepatology* 2002; **35**: 1485-1493

33 **Bedossa P**, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; **24**: 289-293

34 **Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474

35 **Zen Y**, Katayanagi K, Tsuneyama K, Harada K, Araki I, Nakanuma Y. Hepatocellular carcinoma arising in non-alcoholic steatohepatitis. *Pathol Int* 2001; **51**: 127-131

36 **Shimada M**, Hashimoto E, Taniai M, Hasegawa K, Okuda H, Hayashi N, Takasaki K, Ludwig J. Hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. *J Hepatol* 2002; **37**: 154-160

37 **Mori S**, Yamasaki T, Sakaida I, Takami T, Sakaguchi E, Kimura T, Kurokawa F, Maeyama S, Okita K. Hepatocellular carcinoma with nonalcoholic steatohepatitis. *J Gastroenterol* 2004; **39**: 391-396

38 **Yoshioka Y**, Hashimoto E, Yatsuji S, Kaneda H, Taniai M, Tokushige K, Shiratori K. Nonalcoholic steatohepatitis: cirrhosis, hepatocellular carcinoma, and burnt-out NASH. *J Gastroenterol* 2004; **39:** 1215-1218
39 **Cuadrado A**, Orive A, García-Suárez C, Domínguez A, Fernández-Escalante JC, Crespo J, Pons-Romero F. Non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma. *Obes Surg* 2005; **15**: 442-446
40 **McCullough AJ**. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. *Clin Liver Dis* 2004; **8**: 521-533, viii
41 **Jansen PL**. Non-alcoholic steatohepatitis. *Eur J Gastroenterol Hepatol* 2004; **16**: 1079-1085
42 **Sanyal AJ**. AGA technical review on nonalcoholic fatty liver disease. *Gastroenterology* 2002; **123**: 1705-1725
43 **Neuschwander-Tetri BA**, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; **37**: 1202-1219
44 **Ohata K**, Hamasaki K, Toriyama K, Matsumoto K, Saeki A, Yanagi K, Abiru S, Nakagawa Y, Shigeno M, Miyazoe S, Ichikawa T, Ishikawa H, Nakao K, Eguchi K. Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. *Cancer* 2003; **97**: 3036-3043
45 **Kumar D**, Farrell GC, Kench J, George J. Hepatic steatosis and the risk of hepatocellular carcinoma in chronic hepatitis C. *J Gastroenterol Hepatol* 2005; **20**: 1395-1400

S- Editor Zhu LH **L- Editor** Kumar M **E- Editor** Liu Y