Research Article

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Baseline gamma-glutamyl transferase levels strongly correlate with hepatocellular carcinoma development in non-cirrhotic patients with successful hepatitis C virus eradication

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Background & Aims: Hepatitis C virus (HCV)-infected patients with cirrhosis remain at risk of hepatocellular carcinoma (HCC) even after achieving sustained virological response (SVR). The aim of the study was to explore the incidence and risk for HCC among non-cirrhotic patients with an SVR.

Methods: A total of 642 patients with an SVR after peginterferon/ribavirin therapy were enrolled with a median follow-up period of 53.0 months (range: 6–133 months).

Results: Thirty-three of the 642 (5.1%) patients developed HCC over 2324.8 person-years of follow-up. Cox regression analysis revealed that the strongest predictive factor of HCC occurrence was liver cirrhosis (HR 4.98, 95% CI 2.32–10.71, p <0.001), followed by age (HR 1.06, 95% CI 1.02–1.11, p = 0.005) and γGT levels (HR 1.008, 95% CI 1.004–1.013, p <0.001). The incidence of HCC did not differ between patients with high and low baseline γGT levels among patients with cirrhosis (p = 0.53), but the incidence of HCC was significantly higher in non-cirrhotic patients with high γGT levels compared with those with low γGT levels (p = 0.001). Cox regression analysis revealed that the strongest factors associated with HCC development in non-cirrhotic sustained responders were baseline γGT levels (HR 6.44, 95% CI 2.20–18.89, p = 0.001) and age (HR 3.68, 95% CI 1.33–10.17, p = 0.012). The incidence of HCC was not different between older non-cirrhotic patients with high γGT levels and cirrhotic patients (p = 0.34).

Conclusions: HCC remains a threat in non-cirrhotic patients with an SVR. Serum γGT levels helped to identify potential patients at high risk.

Keywords: γGT; HCV; HCC; Treatment; SVR.

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; APRI, the aspartate aminotransferase-to-platelet ratio index; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IL-28B, interleukin 28B; γGT, gamma-glutamyl transferase; SVR, sustained virological response.

Introduction

Hepatitis C virus (HCV) infection has an increasing prevalence worldwide and is one of the leading risks of hepatocellular carcinoma (HCC) [1,2]. As a consequence, the incidence of HCV-related HCC has increased throughout the world in recent decades [3,4]. HCV related HCC occurs predominantly in patients with advanced liver fibrosis [5]. The achievement of a sustained virological response (SVR) through interferon-based therapy has slowed the progression of liver fibrosis [6], which in turn has significantly reduced the development of HCC and liver-related mortality [7–9]. Notably, however, HCC can occur even in patients who demonstrate successful viral eradication [10,11], despite the achievement of an SVR, which demonstrates a durability of >97% during long-term follow-up [12]. The presence of liver cirrhosis before antiviral therapy has been recognized as a potential risk for HCC in patients with an SVR after treatment [10,13]. Whether there is an increased risk for HCC development and the potential risk factors for HCC occurrence among non-cirrhotic patients after successful antiviral therapy remain unclear.

Gamma-glutamyl transferase (γGT), a surrogate of oxidative stress, has been identified as an unfavorable treatment predictor for interferon-based anti-HCV therapy [14–17]. In addition, a

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higher γGT level has been shown to be associated with more advanced liver disease [18,19] and fibrosis progression [20]. However, its role in hepatocarcinogenesis remains conflicting [20–22], and the association between the γGT level and the development of HCC in patients with successful viral eradication has not been assessed. On the other hands, as interleukin 28B (IL-28B) genetic variants have been the most important host factor associated with HCV treatment [23–25], its influence in HCC development remains unclear [26,27]. The current study aimed to elucidate the incidence of HCC by prospectively following an HCV cohort who achieved an SVR. Furthermore, the current study focused on exploring the potential non-invasive surrogate markers, including host genetics and serum γGT, for HCC development after achieving an SVR in non-cirrhotic patients compared with cirrhotic patients.

Materials and methods

The patients were selected from a prospective cohort for a multidisciplinary program of a long-term follow-up study on liver disease at one tertiary hospital and two core regional hospitals from 2002 to 2012. A total of 642 consecutive, pretreatment biopsy-proven chronic hepatitis C patients who achieved an SVR after interferon-based therapy were recruited in the current study, including 556 non-cirrhotic and 86 cirrhotic patients based on pretreatment liver biopsy. An SVR was defined as seronegativity of HCV RNA (Cobas Amplicor Hepatitis C Virus Test, V.2.0; Roche Diagnostics, Branchburg, New Jersey, USA; detection limit: 50 IU/ml) throughout a 24-week post-treatment follow-up period. The patients were excluded if they met any of the following criteria: HIV infection, concurrent hepatitis B virus infection, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, Wilson disease, alcohol abuse (⩾20 g daily). The patients were also excluded if they had evidence of hepatocellular carcinoma before, during or within 6 months post-antiviral therapy. We advised all patients not to drink alcohol except on infrequent social occasions. Patients who drank more than 80 g/day of alcohol for >3 consecutive months during the follow-up period were excluded (Supplementary Fig. 1). All patients provided written informed consent. The institutional review board at each hospital approved the protocols, which conformed to the guidelines of the International Conference on Harmonization for Good Clinical Practice.

Biochemical tests and complete blood counts were performed using a standard autoanalyzer. HCV genotypes were determined using the method described by Okamoto *et al.* [28]. Liver biopsy was performed within 6 months before starting antiviral therapy, and liver histology was graded and staged according to the scoring system described by Knodell and Scheuer [29] by a single pathologist who was blind to the treatment. After achieving an SVR, cirrhotic patients were followed at least every 3 months, and non-cirrhotic patients were followed at least every 6 months; patients were followed more frequently as required for at least 6 months. The person-years estimation was determined from the date of 24 weeks post-treatment to the last visit date at an outpatient clinic if no HCC events were detected or to the date of HCC occurrence. The diagnosis of HCC was based on histological confirmation or imaging criteria according to the recommendations of the American Association for the Study of Liver Disease (AASLD) [30] and the Asian Pacific Association for the Study of the Liver (APASL) [31]. IL-28B rs8099917 was selected as the candidate single nucleotide polymorphism based on our previous studies [23–25,32].

Statistical analyses

Frequency was compared between groups using the χ^2 test, with the Yates correction, or Fisher's exact test. Group means (presented as the mean ± standard deviation) were compared using analysis of variance and Student's *t* test or the non-parametric Mann-Whitney test, when appropriate. The aspartate aminotransferase (AST)-to-platelet ratio index (APRI) was calculated by the following equation: (AST level/upper limit of normal range)/platelet counts (10^9/L) × 100. Kaplan–Meier analysis and the log-rank test were performed by comparing the differences of the cumulative incidence of HCC between determinants. The risk factors independently associated with HCC development were evaluated using Cox regression analysis. The statistical analyses were performed using the SPSS 12.0 statistical package (SPSS, Chicago, IL, USA). All statistical analyses were based on two-sided hypothesis tests with a significance level of $p < 0.05$.

Results

Patient profile

The median follow-up period of the cohort was 53.0 months (range: 6–133 months). Table 1 shows the demographic, clinical, and virological features at baseline for non-cirrhotic and cirrhotic HCV patients on antiviral therapy. Compared with patients without liver cirrhosis, cirrhotic patients were significantly older, had lower platelet counts, lower albumin levels, and a higher APRI and post-treatment γGT levels, and had a higher proportion of diabetes. The IL-28B genotype distribution did not differ between patients with or without liver cirrhosis.

Cumulative incidence of HCC and risk factors for HCC development after achieving an SVR

Thirty-three of the 642 (5.1%) patients developed HCC over 2834.9 person-years of follow-up (annual incidence rate: 1.16%). The cumulative incidence rates of HCC among the patients were 0.5%, 2.7%, and 5.8% at the first, third, and fifth years of follow-up, respectively. Cox regression analysis revealed that the strongest predictive factor for HCC was liver cirrhosis (hazard ratio [HR] 4.98, 95% confidence interval [CI] 2.32–10.71, $p < 0.001$), followed by age (HR 1.06, 95% CI 1.02–1.11, $p = 0.005$) and baseline γGT levels (HR 1.008, 95% CI 1.004–1.013, $p < 0.001$).

The 1-, 3-, and 5-year cumulative incidence rates of HCC were 1.2%, 12.2%, and 22.6%, respectively, for patients with liver cirrhosis compared with 0.4%, 1.3%, and 3.2% for patients without cirrhosis (HR 6.91, 95% CI 3.49–13.69, $p < 0.001$, Fig. 1A). Age over 60 years has been the factor independently associated with HCC development in another cohort of Taiwanese patients with an SVR [10]. The 1-, 3-, and 5-year cumulative incidence rates of HCC in the current study were 0.6%, 4.5%, and 10.2%, respectively, for patients aged ⩾60 years compared with 0.4%, 2.1%, and 4.2% for patients aged <60 years (HR 3.25, 95% CI 1.64–6.44, $p < 0.001$, Fig. 1B).

The baseline γGT levels were significantly higher in patients with HCC than in patients without HCC (94.2 ± 73.7 U/L *vs.* 64.1 ± 56.5 U/L, $p = 0.03$). We further stratified patients into those with and those without a high baseline γGT level using the 75th percentile of the population (75 U/L) as the cut-off value. The 1-, 3-, and 5-year cumulative incidence rates of HCC were 0.6%, 4.5%, and 8.8%, respectively, for patients with a high γGT level compared with 0.4%, 2.1%, and 4.7% for patients without a high baseline γGT level (HR 2.80, 95% CI 1.41–5.53, $p = 0.002$, Fig. 1C). The carriage of favorable rs8099917 TT genotype or unfavorable TG/GG genotype did not influence HCC occurrence either in cirrhotic patients ($p = 0.22$) or in non-cirrhotic patients ($p = 0.36$).

Baseline γGT levels and HCC development after achieving an SVR in patients with and without liver cirrhosis

Among cirrhotic patients, the baseline γGT levels did not differ between patients with and without HCC (80.1 ± 63.2 U/L *vs.* 74.9 ± 65.0 U/L, $p = 0.77$). Neither the percentage of patients with HCC (20.0% *vs.* 17.9%, $p = 0.81$) nor the cumulative incidence of HCC differed between cirrhotic patients with and without a high baseline γGT level ($p = 0.53$, Fig. 2A). By contrast, the baseline γGT levels were significantly higher in non-cirrhotic patients