Pretreatment glucose status determines HCC development in HCV patients with mild liver disease after curative antiviral therapy

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Abstract
Although diabetes mellitus (DM) is known to increase the risk of hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC), the impact of dynamic glucose status on HCC occurrence in chronic hepatitis C (CHC) patients receiving antiviral therapy is unclear. In total, 1112 biopsy-proven patients treated with peginterferon/ribavirin were enrolled in this study. Both pretreatment and post-treatment glucose status, including 75g oral glucose tolerance test (OGTT), were measured to evaluate the association between glucose status and the development of HCC. Of the 1112 patients evaluated, 93.8% developed HCC >5183.8 person-years of follow-up (annual incidence rate: 1.79%). DM only influenced the risk of developing CC in patients with mild liver disease (F0-2) and a sustained virological response (SVR) but not in other patient subpopulations. Cox-regression analysis demonstrated that the strongest factor associated with HCC in patients with mild liver disease and SVR was the presence of DM (hazard ratio [HR]/95% confidence intervals [CI]): 3.79/1.420–10.136, P=0.008), followed by age (HR/CI: 1.06/1.001–1.117, P=0.046) and platelet count (HR/CI: 0.989/0.979–1.000, P=0.05). The percentages of SVR patients with F0-2 with normoglycemia, pre-DM, sub-DM (pre-sDM), and DM before treatment were 45.3% (n=267), 29.9% (n=176), 15.6% (n=92), and 9.2% (n=54), respectively. The percentages of HCC in patients with normoglycemia, pre-sDM, and DM were 1.1%, 3.7%, and 11.1%, respectively (trend P<0.001). Sixteen of the 19 (84.2%) HCC patients possessed glucose abnormality (including 6 patients with DM and 10 patients with pre-sDM) before antiviral therapy. Compared to patients with normoglycemia, the incidence of HCC increased gradually from pre-sDM (HR: 3.6, P=0.05) to DM (HR: 11.6, P=0.001) (adjusted trend P=0.004). We concluded that DM is a critical determinant for the development of HCC in SVR patients with mild liver disease. Pre-sDM status carried an additional risk for HCC, and these patients should also be carefully monitored for HCC after viral eradication.

Abbreviations: ALT = alanine aminotransferase, APRI = the aspartate aminotransferase-to-platelet ratio index, AST = aspartate aminotransferase, DM = diabetes mellitus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, per-DM = pre-diabetics, r-GT = gamma-glutamyl transferase, sub-DM = subclinical diabetics, SVR = sustained virological response.

Keywords: DM, HCC, HCV, OGTT, Pre-DM, SVR, treatment
1. Introduction

Several genetic and environmental factors as well as occupational exposure to carcinogenic toxic substances may lead to hepato-cellular carcinoma (HCC).[11] Hepatitis C virus (HCV) infects ~180 million people, and it is one of the leading causes of HCC worldwide. The seroprevalence of HCV infection has been increasing over the past few decades,[2,3] and as a consequence, the burden of HCC-related to HCV has been growing more rapidly than other etiologies of liver disease.[16,17]

There is a strongly mutual linkage between HCV and diabetes mellitus (DM), and DM is an important feature of the extrahepatic manifestations of HCV infection.[15] The presence of DM determines the disease activity, disease course, and clinical outcomes of HCV.[7] Although the association of DM with HCC is controversial, particularly in hepatitis B virus (HBV) and HCV endemic areas,[6,9] recent meta-analyses have clearly demonstrated that CHC patients with DM carry a higher risk of developing HCC than those without DM.[11] There is also emerging evidence that certain antidiabetic drugs may modulate the risk of HCC development.[10] Based on the causal relationship between DM and HCV, it has been suggested that the contributory risk of DM for HCC development is higher in patients with chronic hepatitis C (CHC) than HBV-infected subjects or those without HBV and HCV infections.[12]

On the other hand, glucose status is associated with the antiviral treatment outcome of CHC infection. The presence of DM or insulin resistance is regarded as an unfavorable predictor of treatment efficacy in patients receiving interferon-based therapy.[13] Meanwhile, elevations in glucose status may be ameliorated after curative antiviral therapy.[14,15] Although successful HCV eradication with antiviral therapy is known to reduce the risk of HCC in patients with all stages of liver disease,[16,17] the effect of DM in HCC among HCV treatment cohorts is less clear.[18] Importantly, it has been demonstrated that prediabetes increases the risk of cardiovascular disease.[19] The risk of HCC development in CHC patients with different glucose statuses is unknown, and the impact of glucose status amelioration on HCC occurrence after antiviral treatment has never been explored. Therefore, we evaluated the influence of pre- and post-treatment glucose status on HCC development after longitudinal follow-up in a large cohort of CHC patients who had received antiviral therapy.

2. Methods

CHC patients receiving antiviral therapy (either peginterferon alfa-2a or peginterferon alfa-2b plus ribavirin) were consecutively recruited as a prospective follow-up cohort at 1 tertiary hospital and 2 core regional hospitals from 2001 to 2012. Patients were excluded if they were coinfected with HIV or HBV. Patients were also excluded if they abused alcohol (≥20g daily) or had evidence of HCC before, during, or within 6 months postantiviral therapy.

Patients who did or did not achieve a sustained virological response (SVR), defined as seronegativity for HCV RNA throughout a 24-week post-antiviral treatment follow-up period, were evaluated further for the risk of HCC development. The post-treatment follow-up strategy was based on cirrhotic status and treatment outcome, as previously described.[16] In general, patients were followed up at least every 3 months if they had advanced liver disease or did not achieve SVR and at least every 6 to 12 months if they had mild liver disease and achieved SVR. The diagnosis of HCC was confirmed by histology or on the basis of image and laboratory evidence, as defined by the American Association for the Study of Liver Diseases[20] and Asian Pacific Association for the Study of the Liver[21] guidelines.

Serum HCV RNA was detected using qualitative real-time polymerase chain reaction (PCR) (COBAS AMPLICOR Hepatitis C Virus Test, ver. 2.0; Roche, Branchburg, NJ; detection limit: 50 IU/mL) and quantification branched DNA assay (Versant HCV RNA 3.0, Bayer, Tarrytown, NY; quantification limit: 615 IU/mL) before 2011. The HCV genotypes were determined using the Okamoto method before 2011.[22] Both HCV RNA and genotype were determined using real-time PCR assay (Real Time HCV; Abbott Molecular, Des Plaines, IL; detection limit: 12 IU/mL) since 2011.[23] Liver disease severity was evaluated by liver biopsy, and histology was graded and staged according to the scoring system described by Scheuer.[24] To prevent the potential pitfall of sampling variability in liver biopsy, we evaluated the association of the risk factors with HCC by stratifying patients according to disease severity: mild (F0-2) or advanced (F3-4).[7] All patients provided written informed consent. The institutional review board at the participating hospital approved the protocols, which conformed to the guidelines of the International Conference on Harmonization for Good Clinical Practice.

2.1. Definition of glucose status

DM history and coadministration of oral hypoglycemic agents or insulin were reviewed by the physicians and recorded by trained coordinators in the outpatient department before, during, and after antiviral therapy. Because glucose abnormalities might be underestimated by measuring fasting plasma glucose (FPG) alone, particularly in CHC patients,[23] 75-g oral glucose tolerance tests (OGTT) were performed in patients without DM history before and 6 months after completing treatment, as previously described.[15,26] The judgment of glucose abnormality was based on the definition established by the American Diabetes Association.[27] Briefly, patients were categorized as having known DM if FPG levels were >126 mg/dL or HbA1C was >6.5% at least twice in the medical record, if there was a previously established diagnosis of DM, or if the patient was currently taking any form of hypoglycaemic drugs or insulin injections. Impaired fasting sugar (IFG) was diagnosed if the FPG was between 100 and 125 mg/dL. Impaired glucose tolerance (IGT) was diagnosed according to a 2-hour plasma glucose concentration of 140 to 199 mg/dL. Patients with IFG, IGT, or HbA1C between 5.7% and 6.4% were defined as cases of prediabetes (pre-DM). For patients without known DM, subclinical DM (Sub-DM) was diagnosed if they met DM criteria with OGT Tresult (2-hplasma glucose concentration of ≥200 mg/dL).

2.2. Statistical analyses

Frequency was compared between groups using the χ² 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 nonparametric Mann–Whitney test when appropriate. 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). All statistical analyses were based on 2-sided hypothesis tests with a significance level of P < 0.05.
### 3. Results

#### 3.1. Patient profile

A total of 1112 patients were enrolled in the current study, with a median follow-up period of 55.9 months (range: 6–142 months). The demographic, clinical, and virological features at baseline are shown in Table 1. The mean age of the patients was 52.1 years, and 52.8% of the patients were male. A total of 397 (35.7%) patients had advanced liver fibrosis (F34), and 863 (77.6%) achieved SVR after antiviral therapy.

#### 3.2. Risk factors for HCC development

Of the 1112 patients analyzed, 93 (8.4%) developed HCC >183.8 person-years of follow-up (annual incidence rate: 1.79%). Patients who developed HCC were older and had a higher incidence of advanced liver fibrosis; a lower SVR rate; lower platelet counts; and higher levels of aspartate aminotransferase (AST), r-glutamyl transferase (r-GT) and alpha-fetoprotein (AFP). Cox-regression analysis revealed that the independent factor most strongly associated with HCC in the treatment cohort was advanced liver disease (hazard ratio [HR]: 3.22/1.59–5.298, \( P < 0.001 \)), followed by non-SVR (HR/CI: 2.23/1.462–3.394, \( P < 0.001 \)), old age (HR/CI: 1.04/1.013–1.061, \( P = 0.002 \)), low platelet count (HR/CI: 0.993/0.989–0.998, \( P = 0.006 \)), and high r-GT (HR/CI: 1.004/1.002–1.007, \( P = 0.001 \)) and AFP levels (HR/CI: 1.002/1.001–1.004, \( P = 0.002 \)) (Table 1). DM was not a risk factor for developing HCC after adjusting for other potential confounders.

#### 3.3. Role of DM in HCC development in patients with differing liver disease severity and treatment outcome

Because advanced liver fibrosis and failure to attain SVR were the major determinants for HCC, we further analyzed the association between DM and HCC by stratifying patients according to these 2 major risk factors. As shown in Fig. 1, DM influenced the occurrence of HCC in patients with mild liver disease (F0-2) and SVR but not the other 3 subpopulations examined. For SVR patients with mild liver disease who had DM, the 1-, 3-, and 5-year cumulative incidence rates of HCC were 0%, 2.8%, and 11.7%, respectively, whereas the cumulative incidence rates for patients without DM were 0.2%, 1.3%, and 1.9%, respectively (HR 5.2, 95% CI: 1.97–13.69, \( P < 0.001 \)). Cox regression analysis revealed that the strongest predictive factor for HCC in SVR patients with mild liver disease was the presence of DM (HR/CI: 3.79/1.420–10.136, \( P = 0.008 \)), followed by age (HR/CI: 1.06/1.001–1.117, \( P = 0.046 \)) and platelet count (HR/CI: 0.989/0.979–1.000, \( P = 0.05 \)) (Table 2).

#### 3.4. Influence of pretreatment and post-treatment glucose status in HCC development in SVR patients with mild liver disease

DM has a significant impact on HCC development in SVR patients with mild liver disease. We further explored the association of dynamic change in glucose status with HCC occurrence in this population. The proportions of patients with normoglycemia, pre-DM, sub-DM (pre-sDM), and DM before treatment were 45.3% (n = 267), 29.9% (n = 176), 15.6% (n = 92), and 9.2% (n = 54), respectively. The proportions of HCC in patients with normoglycemia, pre-sDM, and DM before treatment were 1.1%, 3.7%, and 11.1%, respectively (trend \( P < 0.001 \)). Sixteen of 19 (84.2%) HCC patients possessed glucose abnormalities (including 6 patients with DM and 10 patients with pre-DM) before antiviral therapy. Compared to patients with normoglycemia, the incidence of HCC increased gradually from pre-sDM (HR: 3.6, \( P = 0.05 \)) to DM (HR: 11.6, \( P = 0.001 \)) (adjusted trend \( P = 0.004 \)) (Table 3 and Fig. 2A).

In total, 539 of the 589 (91.5%) patients had post-treatment glucose status information available. Of these patients, the proportions with normoglycemia, pre-DM, pre-sDM, and DM were 62.3% (n = 356), 20.6% (n = 111), 6.9% (n = 37), and 10.2% (n = 55), respectively. The rates of HCC in patients with normoglycemia, pre-sDM, and DM after treatment were 2.1%, 3.4%, and 10.9%, respectively (trend \( P = 0.003 \)). Compared to normoglycemic patients, patients with DM were at significantly higher risk for developing HCC (HR/CI: 5.8/1.951–17.302, \( P = 0.002 \)). However, based on post-treatment glucose status, the risk of HCC did not differ between normoglycemia and pre-sDM patients (Table 3 and Fig. 2B). Although patients were divided

### Table 1

Factors associated with HCC development of the entire cohort.

|                | All patients (n = 1112) | Non-HCC (n = 1019) | HCC (n = 93) | \( P \) | HR | CI               |
|----------------|------------------------|--------------------|--------------|-------|----|------------------|
| Age, y (mean ±SD) | 52.1 ± 11.5            | 51.5 ± 11.5        | 58.3 ± 8.7   | <0.001 | 1.04 | 1.012–1.059     |
| Male gender, n (%) | 587 (52.8)             | 532 (52.2)         | 55 (59.1)    | 0.20  |    |                  |
| Body weight, kg (mean ±SD) | 65.5 ± 11.5           | 65.3 ± 11.5        | 67.7 ± 11.4  | 0.06  |    |                  |
| Fibrosis34, n (%)  | 397 (35.7)             | 331 (32.5)         | 66 (71.0)    | <0.001 | 2.93 | 1.764–4.869     |
| Non-SVR, n (%)    | 249 (22.4)             | 209 (20.5)         | 40 (43.0)    | <0.001 | 2.22 | 1.451–3.403     |
| DM, n (%)         | 161 (14.5)             | 136 (13.3)         | 25 (26.9)    | <0.001 |    |                  |
| Platelet count (>10^12/uL, mean ±SD) | 164 ± 61              | 167 ± 61           | 123 ± 42     | <0.001 | 0.993 | 0.989–0.998     |
| AST, IU/L (mean ±SD) | 381 ± 410             | 378 ± 410          | 414 ± 470    | 0.43  |    |                  |
| r-GT, U/L (mean ±SD) | 65.8 ± 55.0           | 63.0 ± 53.0        | 97.5 ± 74.5  | <0.001 | 1.004 | 1.002–1.007     |
| ALT, IU/L (mean ±SD) | 104 ± 62              | 102 ± 61           | 124 ± 70     | 0.001 |    |                  |
| α-fetoprotein, ng/mL (mean ±SD) | 153 ± 97            | 153 ± 97           | 154 ± 94     | 0.91  |    |                  |
| HCV genotype 1, n/N (%) | 637 (57.7)           | 581 (57.9)         | 56 (60.2)    | 0.61  |    |                  |
| HCV RNA (log IU/mL, mean ±SD) | 5.38 ± 0.96           | 5.38 ± 0.97        | 5.39 ± 0.92  | 0.95  |    |                  |

Hazard ratio of HCC for age (per year increase), F34 (yes vs no), platelet (per ×10^12/uL increase) and α-fetoprotein (per 1 ng/mL increase), ALT=alanine aminotransferase, AST=aspartate aminotransferase, CI=confidence intervals, DM=diabetes mellitus, HCC=hepatocellular carcinoma, HCV=hepatitis C virus, HR=hazard ratio, r-GT=r-glutamyl transferase, SD=standard deviation, SVR=sustained virological response.

* Data available in 1104 patients.
into normoglycemia, pre-sDM, and DM, the differences in glucose status did not impact HCC development in the other 3 subpopulations (SVR & F34, non-SVR & F0–2, and non-SVR & F34) (Supplementary Figure 1, http://links.lww.com/MD/B114).

3.5. Influence of glucose augmentation in HCC

Of the 236 patients who were normoglycemic before treatment, the percentages of normoglycemic, pre-sDM, and DM patients after treatment were 74.6% (n = 176), 25.0% (n = 59), and 0.4% (n = 1), respectively. Of the 249 patients with pre-sDM before treatment, the percentages of normoglycemic, pre-sDM, and DM patients after treatment were 64.3% (n = 160), 35.7% (n = 89), and 0%, respectively. In total, the percentages of non-DM patients with improved, stable, and worse glucose status were 33.0% (n = 160), 54.6% (n = 265), and 12.4% (n = 60), respectively. The rates of HCC in non-DM patients with improved, stable, and worse glucose status were 2.5%, 3.0%, and 0%, respectively. Of the 249 patients with pre-sDM before treatment, the percentages of normoglycemic, pre-sDM, and DM patients after treatment were 64.3% (n = 160), 35.7% (n = 89), and 0%, respectively. In total, the percentages of non-DM patients with improved, stable, and worse glucose status were 33.0% (n = 160), 54.6% (n = 265), and 12.4% (n = 60), respectively. The rates of HCC in non-DM patients with improved, stable, and worse glucose status were 2.5%, 3.0%, and 0%.

Figure 1. HCC development in patients stratified by liver disease severity and SVR status. HCC = hepatocellular carcinoma, SVR = sustained virological response.

### Table 2
Factors associated with HCC development in SVR patients with mild liver disease (F0–2).

|                          | All patients (n = 589) | Non-HCC (n = 570) | HCC (n = 19) | P     | HR   | CI         | P     |
|--------------------------|------------------------|-------------------|-------------|-------|------|------------|-------|
| Age, y (mean ± SD)       | 50.0 ± 11.5            | 49.7 ± 11.5       | 58.1 ± 9.9  | 0.002 | 1.06 | 1.001–1.117| 0.046 |
| Male gender, n (%)       | 326 (55.5)             | 313 (54.9)        | 13 (68.4)   | 0.24  |      |            |       |
| Body weight, kg (mean ± SD) | 64.7 ± 11.2            | 64.7 ± 11.3       | 62.4 ± 11.2 | 0.38  |      |            |       |
| DM, n (%)                | 54 (9.2)               | 48 (8.4)          | 6 (31.6)    | 0.005 | 3.79 | 1.420–10.136| 0.008 |
| Platelet count (× 10^3/uL, mean ± SD) | 180 ± 54               | 181 ± 54          | 143 ± 44    | 0.002 | 0.989| 0.979–1.000 | 0.05  |
| Ferritin, ng/mL (mean ± SD) | 366 ± 299              | 351 ± 300         | 514 ± 608   | 0.10  |      |            |       |
| r-GT, U/L (mean ± SD)    | 59.2 ± 52.7            | 58.7 ± 52.4       | 74.6 ± 88.8 | 0.20  |      |            |       |
| AST, IU/L (mean ± SD)    | 101 ± 63               | 100 ± 62          | 126 ± 87    | 0.07  |      |            |       |
| ALT, IU/L (mean ± SD)    | 161 ± 103              | 160 ± 100         | 193 ± 48    | 0.16  |      |            |       |
| α-fetoprotein, ng/mL (mean ± SD) | 10.7 ± 29.5         | 10.5 ± 29.4       | 17.8 ± 32.1 | 0.30  |      |            |       |
| HCV genotype 1, n/N (%)  | 312 (53.3)             | 303 (53.5)        | 9 (47.4)    | 0.60  |      |            |       |
| HCV RNA (log IU/mL, mean ± SD) | 5.31 ± 0.99           | 5.31 ± 0.99       | 5.18 ± 0.96 | 0.57  |      |            |       |
| F2, n (%)                | 258 (43.8)             | 246 (43.2)        | 12 (63.2)   | 0.02  |      |            |       |

Hazard ratio of HCC is for age (per year increase), platelet (per × 10^3/uL, increase) and DM (Yes versus No), ALT = alanine aminotransferase, AST = aspartate aminotransferase, CI = confidence intervals, DM = diabetes mellitus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, HR = hazard ratio, r-GT = r-glutamyl transferase, s-GT = s-glutamyl transferase, SD = standard deviation, SVR = sustained virological response.

*Data available in 585 patients.
respectively \((P=0.49)\), and the incidence of HCC did not differ among the 3 groups \((P=0.36\), Supplementary Figure 2, http://links.lww.com/MD/B114\).

4. Discussion

The association of DM with HCC has been widely discussed. To our knowledge, our study is the first to explore the impact of dynamic changes in glucose status on HCC occurrence in CHC patients receiving antiviral therapy. Here, we demonstrated that DM is a major risk factor for HCC occurrence among patients with mild liver fibrosis despite the benefit of viral eradication. Notably, prediabetic status may also carry some risk for developing HCC. Most of the SVR patients with mild liver disease who developed HCC had a glucose abnormality prior to HCV eradication, even if the glucose status was ameliorated by curative antiviral therapy.

CHC patients with glucose abnormalities are generally older, have more advanced liver disease, and are prone to experience treatment failure. Because these factors are critical determinants for HCC development, they may confound the association of DM with HCC in patients receiving antiviral therapy. Therefore, the magnitude of the effect of DM on the chance of developing HCC may have been masked by other potential risk factors after statistical adjustment.\(^{16,28}\) However, DM does increase the risk of HCC development in patients when restricted to the low-risk population. Although the incidence of HCC is low in SVR patients with mild liver disease, HCC does occur in a minority of patients upon long-term follow-up. We identified DM as the most critical risk factor for developing HCC in these patients, with HCC risk being ~4-fold greater in diabetes patients than in nondiabetic patients. Similarly, Hung et al cautioned that HCC occurrence may be increased in noncirrhotic patients who have achieved SVR.\(^{18}\) Almost all DM patients experience the prediabetes condition (i.e., IFG and/or IGT) before a definitive diagnosis is confirmed. In addition to developing DM, the prediabetes condition has been suggested to carry a risk of cardiovascular disease.\(^{1,18}\) Therefore, we aimed to determine if prediabetes similarly impacted HCC. We found a trend between HCC development and the transition from normoglycemia to DM. Insulin resistance, regardless of DM status, has been linked with HCV-related HCC,\(^{29}\) implying the potential oncogenic role of hyperglycemia in hepatocarcinogenesis. Although the pathophysiological mechanism remains unclear, insulin resistance might influence hepatocarcinogenesis via several molecular pathways, such as phosphatase and tensin homolog (PTEN)/P13K/Akt and MAP kinase (MAPKK).\(^{30}\) Glucose abnormality might also be related to dysregulation of the insulin-like growth factor (IGF) system and the type-IIGF receptor (IGF-IR) signaling pathway, which is important for HCC development.\(^{31}\) The knowledge of these pathways has important consequences in the goal of HCC treatment.\(^{32-35}\)

Table 3

| Pretreatment | HCC n/N (%) | HCC incidence (per person-year) | HR | CI | \(P\) Unadjusted trend | \(P\) Adjusted trend |
|--------------|-------------|---------------------------------|----|----|----------------------|---------------------|
| Normoglycemia | 3/267 (1.1%) | 0.22\%                          | 1  |    | 0.098–12.390          | 0.05                |
| Pre-sDM      | 10/268 (3.7%)| 0.77\%                          | 3.56|    | 2.055–6.825          | 0.0003              |
| DM           | 6/54 (11.1%) | 2.52\%                          | 11.64|    | 2.905–46.606         | 0.001               |
| Post-treatment | 7/336 (2.1%) | 0.42\%                          | 1  |    | 0.046–4.335          | 0.04                |
| Normoglycemia | 5/148 (3.4%) | 0.62\%                          | 1.44|    | 0.456–4.335          | 0.94                |
| Pre-sDM      | 6/55 (10.9%) | 2.44\%                          | 5.81|    | 1.951–17.302         | 0.002               |

\(CI=\) confidence intervals, DM=diabetes mellitus, HCC=hepatocellular carcinoma, HR=hazard ratio, pre-sDM=pre- and subclinical diabetes, SVR=sustained virological response.

* Post-treatment glucose status was available in 539 (91.5%) patients.

* Adjusted age, platelet counts, and aspartate aminotransferase.
DM is one of the most significant extra hepatic manifestations of HCV infection. Previously, it was shown that the prevalence of glucose abnormalities was 3 times greater in anti-HCV positive patients than in anti-HCV-negative patients. Robust epidemiological evidence has also shown that HCV viremia, but not anti-HCV seropositivity alone, increased the association with type 2 DM. Glucose status might be uncovered in CHC patients. In individuals without a known DM history, CHC patients were at higher risk for developing DM and IGT than controls (odds ratio 3.3). Consistent with our previous reports, without OGTT, only one-tenth had deteriorated sugar after antiviral therapy. Among nondiabetic SVR patients and only one-tenth had deteriorated sugar after antiviral therapy. Notably, improvement in glucose status did not benefit HCC development, suggesting that insulin resistance elicited certain oncogenic processes that were beyond the impact of virus and fibrogenesis. Given the high prevalence of glucose abnormality corresponding to hepatic and extra hepatic long-term outcome in CHC patients, new parameters or cut off levels for defining glucose abnormality for normoglycemia, prediabetes and DM might be warranted. In conclusion, although the likelihood of developing HCC in CHC patients with mild fibrosis is low, it may still occur even after SVR is achieved. A major risk factor for this population is glucose abnormality. Due to their increased risk for HCC, patients with pre-dm should undergo increased surveillance in the post-treatment period.

Acknowledgments
This study was supported by Kaohsiung Medical University Hospital (KMUH99-842, KMUH103-305), and Kaohsiung Medical University “Aim for the Top Universities Grant, grant No. KMU-TP104E08 and KMU-TP104E09” and National Science Council of Taiwan (NSC 103-2314-B-037-055-MY3, MOST 103-2314-B-037-061-MY3).

References
[1] Rapisarda V, Loreto C, Malaguarnera M, et al. Hepatocellular carcinoma and the risk of occupational exposure. World J Hepatol 2016;8:173–90.
[2] Mohd Hanafiah K, Groeger J, Flaxman AD, et al. Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence. Hepatology 2013;57:1333–42.
[3] Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med 2001;345:41–52.
[4] Davila JA, Morgan RO, Shaib Y, et al. Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. Gastroenterology 2004;127:1372–80.
[5] Hirooka A, Hidaka S, Shima Y, et al. Recent trends of Japanese hepatocellular carcinoma due to HCV in aging society. Hepatogastroenterology 2012;59:1893–5.
[6] Huang JF, Dai CY, Hwang SJ, et al. Hepatitis C viremia increases the association with type 2 diabetes mellitus in a hepatitis B and C endemic area: an epidemiological link with virological implication. Am J Gastroenterol 2007;102:1237–43.
[7] Huang CF, Dai CY, Yeh ML, et al. Association of diabetes and PNPLA3 genetic variants with disease severity of patients with chronic hepatitis C virus infection. J Hepatol 2015;62:512–8.
[8] Chen CT, Chen JY, Wang JH, et al. Diabetes mellitus, metabolic syndrome and obesity are not significant risk factors for hepatocellular carcinoma in an HBV- and HCV-endemic area of Southern Taiwan. Kaohsiung J Med Sci 2013;29:451–9.
[9] Tung HD, Wang JH, Tseng PL, et al. Neither diabetes mellitus nor overweight is a risk factor for hepatocellular carcinoma in a dual HBV and HCV endemic area: community cross-sectional and case-control studies. Am J Gastroenterol 2010;105:624–31.
[10] Dyal HK, Aguilar M, Bartos G, et al. Diabetes mellitus increases risk of hepatocellular carcinoma in chronic hepatitis C virus patients: a systematic review. Dig Dis Sci 2016;61:636–45.
[11] Singh S, Singh PP, Singh AG, et al. Anti-diabetic medications and the risk of hepatocellular cancer: a systematic review and meta-analysis. Am J Gastroenterol 2013;108:881–91. quiz 892.
[12] Chen CL, Yang HI, Yang WJ, et al. Metabolic factors and risk of hepatocellular carcinoma by chronic hepatitis B/C infection: a follow-up study in Taiwan. Gastroenterology 2008;135:111–21.
[13] Dai CY, Huang JF, Hsheh MY, et al. Insulin resistance predicts response to peginterferon-alpha/ribavirin combination therapy in chronic hepatitis C patients. J Hepatol 2009;50:712–8.
[14] Huang JF, Dai CY, Yu ML, et al. Pegylated interferon plus ribavirin therapy improves pancreatic beta-cell function in chronic hepatitis C patients. Liver Int 2011;31:1153–60.
[15] Huang JF, Yu ML, Huang CF, et al. The outcomes of glucose abnormalities in pre-diabetic chronic hepatitis C patients receiving peginterferon plus ribavirin therapy. Liver Int 2012;32:962–9.
[16] Huang CF, Yeh ML, Tsai PC, et al. Baseline gamma-glutamyl transferase levels strongly correlate with hepatocellular carcinoma development in non-cirrhotic patients with successful hepatitis C virus eradication. J Hepatol 2014;61:67–74.
[17] Morgan RL, Baack B, Smith BD, et al. Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies. Ann Intern Med 2013;158:329–37.
[18] Hung CH, Lee CM, Wang JH, et al. Impact of diabetes mellitus on incidence of hepatocellular carcinoma in chronic hepatitis C patients treated with interferon-based antiviral therapy. Int J Cancer 2011;128:2344–52.
[19] Kannel WB, McGee DL. Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. Diabetes Care 1979;2:120–6.
[20] Beux J, Sherman M. Management of hepatocellular carcinoma: an update. Hepatology 2011;53:1020–2.
[21] Omata M, Lesmana LA, Tateishi R, et al. Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. Hepatol Int 2010;4:439–74.
[22] Okamoto H, Tokita H, Sakamoto M, et al. Characterization of the genomic sequence of type V (or 3a) hepatitis C virus isolates and PCR primers for specific detection. J Gen Virol 1993;74(Pt 11):2385–90.
[23] Vermehren J, Yu ML, Monto A, et al. Multi-center evaluation of the Abbott RealTime HCV assay for monitoring patients undergoing antiviral therapy for chronic hepatitis C. J Clin Virol 2011;52:133–7.
[24] Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. J Hepatol 1991;13:372–4.
[25] Hung WW, Chang CJ, Lee YJ, et al. Metabolic risk factors in southern Taiwanese with impaired fasting glucose of 100 to 109 mg/dL. Metabolism 2007;56:328–32.
[26] Huang JF, Yu ML, Dai CY, et al. Reappraisal of the characteristics of glucose abnormalities in patients with chronic hepatitis C infection. Am J Gastroenterol 2008;103:1933–40.
[27] Diagnosis and classification of diabetes mellitus. Diabetes Care 2014;37 (Suppl 1):S81–90.
[28] Chang KC, Hung CH, Lu SN, et al. A novel predictive score for hepatocellular carcinoma development in patients with chronic hepatitis C after sustained response to pegylated interferon and ribavirin combination therapy. J Antimicrob Chemother 2012;67:2766–72.
[29] Hung CH, Wang JH, Hu TH, et al. Insulin resistance is associated with hepatocellular carcinoma in chronic hepatitis C infection. World J Gastroenterol 2010;16:2265–71.
[30] Facciorusso A. The influence of diabetes in the pathogenesis and the clinical course of hepatocellular carcinoma: recent findings and new perspectives. Curr Diabetes Rev 2013;9:382–6.
[31] Alexia C, Wallot G, Lasfer M, et al. An evaluation of the role of insulin-like growth factors (IGF) and of type-I IGF receptor signalling in hepatocarcinogenesis and in the resistance of hepatocarcinoma cells against drug-induced apoptosis. Biochem Pharmacol 2004;68:1003–15.
[32] Bertino G, Ardiri A, Malaguarnera M, et al. Hepatocellular carcinoma serum markers. Semin Oncol 2012;39:410–33.
[33] Bertino G, Demma S, Ardiri A, et al. Hepatocellular carcinoma: novel molecular targets in carcinogenesis for future therapies. Biomed Res Int 2014;2013:45693.
[34] Bertino G. Hepatocellular carcinoma: present and future. Chin Clin Oncol 2012;1:14.

[35] Bertino G, Di Carlo I, Ardiri A, et al. Systemic therapies in hepatocellular carcinoma: present and future. Future Oncol 2013;9:1533–48.

[36] Lecube A, Hernandez C, Genesca J, et al. High prevalence of glucose abnormalities in patients with hepatitis C virus infection: a multivariate analysis considering the liver injury. Diabetes Care 2004;27:1171–5.

[37] Huang JF, Yu ML, Dai CY, et al. Glucose abnormalities in hepatitis C virus infection. Kaohsung J Med Sci 2013;29:61–8.