Dear Editor，

Thank you very much for your letter and the reviewers' comments on our manuscript entitled "Type 2 diabetes mellitus characteristics affect hepatocellular carcinoma development in chronic hepatitis B patients with cirrhosis" (Manuscript NO.: 81240, Retrospective Study). We found the reviewers' comments very helpful and constructive and we have revised the manuscript accordingly. Our point-by-point responses to the reviewers' comments are detailed below.

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: Li et al. enrolled 412 CHB patients, including 196 patients with DM and the other 216 non-DM patients to assess the effect of DM on the risk of HCC. They found that DM, male, alcohol abuse, AFP >20 ng/mL and HBsAg >2.0 log IU/mL were risk factors for HCC development. DM duration of more than 5 years, diet control and insulin ± sulphonylurea therapy significantly increased the risk of hepatocarcinogenesis. They concluded that DM greatly impacts the outcome of HBV. Several issues need to be addressed.

Comments: 1. Were all the patients cirrhotic, as described in the materials and methods? If yes, please clarify it throughout the manuscript from title to conclusion.

Reply: Thank you for your kind advice. It has been clarified in the manuscript.

2. How about the HbA1C levels of DM patients? The well vs. poor control of DM might have impacts on the HCC risk.

Reply: Thank you for your advice. HbA1c has been reported to be related

with HCC development [1,2]. It would be meaningful to add the information in the results. However, the HbA1c data were not collected in this study because that many patients were lack of this data. Thus, it will be analyzed and discussed in further independent study.

[1] Mak LY, Hui RW, Lee CH, Mao X, Cheung KS, Wong DK, Lui DT, Fung J, Yuen MF, Seto WK. Glycemic burden and the risk of adverse hepatic outcomes in patients with chronic hepatitis B with type 2 diabetes. Hepatology. 2022 Sep 21.

[2] Tateishi R, Matsumura T, Okanoue T, Shima T, Uchino K, Fujiwara N, Senokuchi T, Kon K, Sasako T, Taniai M, Kawaguchi T, Inoue H, Watada H, Kubota N, Shimano H, Kaneko S, Hashimoto E, Watanabe S, Shiota G, Ueki K, Kashiwabara K, Matsuyama Y, Tanaka H, Kasuga M, Araki E, Koike K; LUCID study investigators. Hepatocellular carcinoma development in diabetic patients: a nationwide survey in Japan. J Gastroenterol. 2021 Mar;56(3):261-273.

3. Did any DM patients receive metformin? A recent large-scale study showed that metformin use greatly reduced the risk of HCC development after HCV eradication [Tsai et al. J Hepatol. 2022 Oct 5:S0168-8278(22)03129-4. doi: 10.1016/j.jhep.2022.09.019. Epub ahead of print. PMID: 36208843.]. Please discuss it.

Reply: In this study, 112 (57.1%) DM patients received metformin (Table 1). Compared to patients who received metformin, insulin ± sulphonylurea therapy (HR, 1.45; 95% CI, 0.26, 7.96; P=0.041) and diet control only (HR, 10.70; 95% CI, 2.91, 39.31; P<0.001) were significantly related with hepatocarcinogenesis in DM group (Table 3). Consistent with the study you mentioned, our results also indicated that metformin treatment had significantly lower risk for HCC in patients. This reference has been cited in discussion (Line 237): A large-scale study also showed that the use of metformin among DM patients can significantly reduce the HCC risk in chronic hepatitis C (CHC) patients. The underlying mechanism has not been

fully understood, but it may be related with the anti-proliferative and immune modulation effect of metformin. Although evidence suggests that sulfonylureas can increase the risk of HCC, we found that the use of metformin with or without sulphonylurea still had significantly lower risk for HCC. The above results suggested that good diabetic management and appropriate therapy are crucial in cirrhotic CHB patients with DM.

4. A recent study showed rGT levels were associated with risk of HCC among HBV patients on NUC therapy [Huang CF, et al. Liver Int. 2022 Jan;42(1):59-68.]. The authors reported the baseline rGT levels among the HBV patients, but did not take it into analysis for HCC risk. Please analyze and discuss it.

Reply: Thank you for your advice. The γ-GTP has been taken into analysis accordingly (Table 2 and 3). The results showed that the level of γ-GTP did not differ between two groups in both univariate and multivariate analysis (*P*=0.692; *P*=0.530). In DM patients, γ-GTP level was also not significantly related to HCC development (*P*=0.762). In the discussion section, we also added the discussion about γ-GTP: A recent study showed that γ-GTP levels were related with the risk of HCC among HBV patients on nucleos(t)ide analogue (NUC) therapy. But in this study, the level of γ-GTP did not differ between two groups in both univariate and multivariate analysis (P=0.692; P=0.530). Thus, whether γ-GTP levels affect the prognosis of CHB patients still needs to be further verified (Line 213).

5. The preparation of NUC (TDF vs ETV) for HBV therapy might have different risks of HCC. How about the NUC used in this study?

Reply: The analysis of HBV therapy has been added accordingly (Supplementary Table 1). We found that the proportion of different HBV therapies between two groups did not differ significantly. Therefore, the HBV therapy may not affect the risk of HCC in this study (Line 147).

Supplementary Table 1. HBV therapy of patients

	DM group with HBV therapy (n=137)	Non-DM group with HBV therapy (n=144)	*P*-value
NUC			
Tenofovir	45 (32.8)	42 (29.2)	0.521
Entecavir	70 (51.1)	62 (43.1)	0.190
others	13 (9.5)	25 (17.4)	0.057
Non-NUC	9 (6.6)	15 (10.4)	0.290

6. DM is highly associated with fatty liver. However, fatty liver was significantly associated with lower cirrhosis and HCC risk in a previous study [Li J, et al. J Infect Dis. 2021 Jul 15;224(2):294-302.]. How about the data in this study? Please discuss it

Reply: Thank you for advice. Non-alcoholic fatty liver disease (NAFLD) and DM regularly co-exist and can act synergistically to drive adverse outcomes [1]. Their coexistence may increase the risks of cirrhosis, HCC and death [2]. But the exact mechanism still remains to be investigated. In this study, the data of fatty liver were not able to be collected. It will be investigated in future independent study.

[1] Hazlehurst JM, Woods C, Marjot T, Cobbold JF, Tomlinson JW. Non-alcoholic fatty liver disease and diabetes. Metabolism. 2016;65(8):1096-1108.

[2] Dowman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. QJM. 2010 Feb;103(2):71-83.

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: This is a very interesting paper. Nevertheless, this is a already highly discussed subject, and it adds little novelty to the literature. Methods are well done, and it is nicely written. I would suggest publication after a good language review - this is the major let down of this paper.

Reply: Thank you for your advice. The language editing has been done by FILIPODIA Editing Service and we have revised the manuscript accordingly. Following is the editing certificate.

filipodia

CONFIDENTIAL
November 23, 2022

Certificate Service Confirmation

To Whom It May Concern,

Filipodia provided comprehensive editing services for **Manuscript NO: 81240** (**Type 2 diabetes mellitus characteristics affect hepatocellular carcinoma development in chronic hepatitis B patients**) by **Li M *et al.***, which is under consideration for publication in your journal. The edit has achieved Grade A: priority publishing; no language polishing required after editing.

Should you require any additional information, please do not hesitate to contact me.

Jennifer C van Velkinburgh, PhD
President and Chief Editor & Writer,
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We appreciate the opportunity to revise our manuscript. We hope that you will now find our revised manuscript acceptable for publication.

Sincerely yours,

Manyu Li

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