Dear Editor,

We are submitting our manuscript as an original article of observational study for publication in World Journal of Hepatology. We thank you for the kind review, and most importantly, the opportunity to revise our manuscript (74876) for World Journal of Hepatology now titled "Prevalence of nonalcoholic fatty liver disease and its association with age in patients with type 2 diabetes mellitus".

We wish to thank the editor and reviewers for their time and invaluable comments to improve our manuscript. We hope that our revised manuscript is now acceptable for publication in your esteemed journal.

Our point-to-point responses to the reviewers are as below.

Sincerely,

Kentaro Yoshioka

Editorial Office's comments

1) Science Editor:

Thank you for the comments. We had revised the manuscript as advised.

Comment #1) Where do clinical and laboratory results come from? Please, explain how data were retrieved in the Methods section.

Response: Data of the patients with diagnosis of T2DM were retrieved from the hospital database.

Comment #2) The diagnosis of NAFLD is a subject of debate. Please, add more explanations on biopsy-free diagnosis and its limitations.

Response: We added the sentences below as limitations.

Second, fatty liver was diagnosed by CT in this study. The sensitivity of MRI, US, and CT for detecting a fatty liver of 5% or higher is 77%−80%, 53%−62%, and 50% compared with liver biopsy (J Hepatol. 2010 Apr; 52(4):579-85.). However, a liver biopsy is invasive and has a risk of severe complications. Thus noninvasive modalities, such as US, CT, and MRI, have been commonly used to detect fatty liver. MRI is expensive and scarce. The disadvantage of US is its subjective nature. The high liver iron content increases CT Hounsfield units and may obliterate the diagnosis of fatty liver. However, CT is widely available in Japan, and the diagnosis is objective. Thus, CT is a promising modality for diagnosing fatty liver.

Comment #3) Why NAFLD decreases with age? Please, try to add some more explanations on this issue. Does NAFLD actually decrease or maybe it does not increase in a certain subpopulation of T2DM? Consider the limitations of a retrospective and transversal study.

Response: We added the sentences below as limitations.

First, it is a cross-sectional study. There may be a question of whether NAFLD decreases with age. Poor nutritional status in older people may be one reason why NAFLD decreases with age. It is also possible that we assessed a certain subpopulation of T2DM with a low risk of NAFLD and survival until older age, while the patients with a high risk of NAFLD dropped out until older age because of complications. Thus, the temporal association of NAFLD with the

factors assessed in this study has to be clarified by prospective cohort studies.

Comment #4) What is the relative importance of NAFLD in T2DM in relation to the whole population? Even not being part of this study, it would be important to have at least an estimate of the prevalence of NAFLD in the general population (in the same area, preferentially).

Response: We added the sentences below to Discussion.

In a preliminary study of 179 subjects who attended health screening in our hospital (120 males and 59 females; 53.7 ± 10.8 years), CT detected fatty liver in 40 (22%). Thus, the prevalence of NAFLD in T2DM is higher than in the general population considering older age.

Reviewer#1

Thank you for the comments. We had revised the manuscript as advised.

1) Abstract: in the methods section the age of patients should be added.

   Response: Age was added.

2) Core tip: this section should be attractive for the readers and not just a summary of the study.

   Response: We revise the core tip to be more attractive.

3) Introduction: a brief overview on NAFLD and its relevant cardiometabolic burden since childhood should be added (e.g.PMIDs 28686220 34629802). More, the intriguing link between NAFLD and T2D needs to be clearer discussed (e.g. PMID: 32165250).

   Response: We added a brief overview on NAFLD and its relevant cardiometabolic burden since childhood to Introduction. We also added a

clearer discussion the intriguing link between NAFLD and T2D.

4) Methods: statistics needs to be clarified (e.g. for categorical variables we used....)

   Response: We clarified the description of statistics.

5) Results: this section needs to be reorganized. As a suggestion, it should be useful to show the results by subdividing the associations according to " blood count parameters", "kidney function", and "metabolic status (lipids)"

   Response: We reorganized the section of results as suggested.

6) Discussion: it needs to be revised. At the beginning, main findings of the study should be mentioned. As cardiovascular risk markers, the relationship with lipids should be considered in the discussion about the association of NAFLD with CVD.

   Response: We mentioned the main findings at the beginning. We mentioned the lipids as cardiovascular risk markers in the discussion about the association of NAFLD with cardiovascular disease.

7) English language needs a substantial revision.

   Response: English language was edited by Enago (www.enago.jp).

Reviewer#2

Thank you for the comments. We had revised the manuscript as advised.

1) The title is very big and with little information in the study.

   Response: We changed the title into “Prevalence of nonalcoholic fatty liver disease and its association with age in patients with type 2 diabetes mellitus”.

2) The author only described the patients are diabetic and no other liver diseases. However, what about those diabetic patients' conditions? Are they newly diagnosed or with certain years of diabetes? Or are those patients with or without complications? Or what treatments are they have at the time of the study? Are they under oral anti-diabetic treatments or with insulin injection or GLP-1R agonist?

   Response: We added the data of conditions of T2DM. We described the durations, complications, and treatments

3) There are too many influence factors in diabetic patients that will affect NAFLD, such as TZD, GLP-1R agonist, metformin, duration of diabetes, so the authors should investigate such in a certain condition of diabetic patients, for example newly diagnosed diabetic patients.

   Response: We added the data of conditions of T2DM. We described the durations, complications, and treatments. There were 322 patients treated with oral hypoglycemic agents, 32 with insulin, one with a glucagon-like peptide-1 receptor agonist, and 82 with diet and exercise. All patients had more than a year with T2DM. The prevalence of NAFLD did not vary according to the methods of treatment.

4) If the patients included have a big variety condition then the results will be wired, like the author said that "Fatty liver was significantly associated with greater height ($p < 0.0001$) " , and there is sex differences in NAFLD, however, the authors should consider the menopausal state of women.

   Response: Unfortunately we have no data on the menopausal state of women.

Reviewer#3

Thank you for the comments. We had revised the manuscript as advised.

1) The limitations section of the study is absent.

   Response: We described the section of three limitations.

2) The indications of CT are not clearly defined; thus the study group consists of diabetic patients with problems requiring imaging. So, the group may not represent all cases with diabetes.

   Response: The CT indications were to screen for diseases in the chest and abdomen in 430 patients. The CT was performed to investigate liver diseases in seven patients. We excluded only 115 participants because of absence of l CT. Thus we think 437 patients studied in the present study represent all cases with T2DM.

3) Histology is the gold standard of steatosis and based on CT alone is not sufficient to determine NAFLD to give the prevalence. The iron level in the liver highly resembles steatosis with CT liver-spleen.

   Response: We added the sentences below as a limitation of the present study.

   Second, fatty liver was diagnosed by CT in this study. The sensitivity of MRI, US, and CT for detecting a fatty liver of 5% or higher is 77%−80%, 53%−62%, and 50% compared with liver biopsy (J Hepatol. 2010 Apr; 52(4):579-85.). However, a liver biopsy is invasive and has a risk of severe complications. Thus noninvasive modalities, such as US, CT, and MRI, have been commonly used to detect fatty liver. MRI is expensive and scarce. The

disadvantage of US is its subjective nature. The high liver iron content increases CT Hounsfield units and may obliterate the diagnosis of fatty liver. However, CT is widely available in Japan, and the diagnosis is objective. Thus, CT is a promising modality for diagnosing fatty liver.

4) The reported specificity is 100% while sensitivity is 80%. (J Hepatol. 2010 Apr; 52(4):579-85.) Thus 20% of cases may be dismissed.

   Response: We added the sentences below as limitations of the present study. Second, fatty liver was diagnosed by CT in this study. The sensitivity of MRI, US, and CT for detecting a fatty liver of 5% or higher is 77%−80%, 53%−62%, and 50% compared with liver biopsy (J Hepatol. 2010 Apr; 52(4):579-85.). However, a liver biopsy is invasive and has a risk of severe complications. Thus noninvasive modalities, such as US, CT, and MRI, have been commonly used to detect fatty liver. MRI is expensive and scarce. The disadvantage of US is its subjective nature. The high liver iron content increases CT Hounsfield units and may obliterate the diagnosis of fatty liver. However, CT is widely available in Japan, and the diagnosis is objective. Thus, CT is a promising modality for diagnosing fatty liver.

5) Statistics: Unnecessary long decimals of p-values could be limited to three numbers as $p<0.001$ is the most significant. Also very low p-values like $p<0.0001$ will be rarely encountered because it would mean that the trial was overpowered and should have had a smaller sample size. It would seem appropriate, therefore, to require investigators to explain such results and to consider rejecting the research involved. (DOI: 10.1515/CCLM.2004.054) In most fields, a value of 0.0001 would be

statistically significant. In a few it would not- e.g. in high-energy physics, where the experiments generate vast amounts of data, the thresholds are set much higher. In medical research where generating this amount of data is impossible, a value like this would be more than enough (indeed many journals will just report a value of "> 0.001" as such) ((https://www.quora.com/Is-a-P-value-of-0-0001-statistically-significant).

Response: P-values were limited to three numbers as $p<0.001$ is the most significant.

6) Minor typo in Table 2. "dyalysis > dialysis

   Response: We correct the miss spell of dialysis in Table 2.

7) Overall, the prevalence in this study has to be reevaluated.

   Response: We mentioned that the prevalence in this study has to be reevaluated by multicenter studies.