Format for ANSWERING REVIEWERS

September 23, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 18324-Review.doc).

Title: Histopathological differences utilizing the NAFLD Activity score criteria in diabetic (T2DM) and non-diabetic patients with NAFLD

Author: Bharat K Puchakayala, Siddharth Verma, Pushpjeet Kanwar, John Hart, Raghavendra R Sanivarapu, Smruti R Mohanty

Name of Journal: *World Journal of Hepatology*

ESPS Manuscript NO: 18324

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

1. Reviewed by 02444959
   Comments to Authors
   This is an excellent work dealing with a very interesting topic, the histopathological alterations in diabetic and non-diabetic patients with non-alcoholic liver disease (NAFLD). There are few studies describing the mentioned differences and this work constitutes a novel approach, although few limitations can be detected (sample size, limited data about the diabetic patients, etc.). However, this study is a nice piece of work that deserves to be published.

   Reply
   Dear Reviewer 02444959,

   We thank you for your supportive remarks. We do acknowledge our limitations of the study namely study size and have added few additional data about diabetic patients especially Hba1c level and few indirect serological markers of liver inflammation as suggested.

2. Reviewed by 00187828

Comments to Authors

The manuscript entitled Histopathological differences utilizing the NAS criteria in diabetic (T2DM) and non-diabetic patients with Nonalcoholic Fatty Liver Disease (NAFLD)by Bharat K Puchakayala et al.,is well-written,presented and illuminatig. The authors showed clearly the impact of T2DM on NAFLD. Comorbidity seems to be very important in converting the diease and determining the severity of the disease either ways.

Reply

Dear Reviewer 00187828,

We have made an attempt to present the differences among diabetic and non-diabetic patients with NAFLD with the available resources at that time. Thank you for your kind support.

3. Reviewed by 00181536

Comments to Authors

This study reveals the histopathological differences of non-alcoholic fatty liver disease (NAFLD) according to diabetic or non-diabetic. The authors found that complicating type2 diabetes was correlated with advanced fibrosis and should be extensively examined for such patients. The results are interesting, but several issues need to be addressed. Major comments 1. The criteria for the diagnosis of type 2 diabetes was too old as used on 2004. This criteria does not include the patients with high HbA1c that is included in the recent criteria (Diabetes Care Vol38, Sup1, 2015). As this article would be published in 2015, the criteria should be updated. 2. As the patients were corrected from 1995 to 2005, the longitudinal follow-up data for the patients should be added even for a part of them. Minimum ten years have passed since the liver biopsy correction. 3. One additional table showing the background data of NASH and NAFL is recommended to add. 4. As ALT is included in the variables in Table 3, the ALT data should be added in Table1. In addition, as AST/ALT ratio is regarded to be one marker to distinguish NAFL and NASH, the AST and the AST/ALT ratio should be added. Other NASH diagnostic marker such as Fib-4 index or APRI score should be added to acquire the patients' characters. Minor Comments 1. Table 1; the units of data should be added.

Reply

Dear Reviewer 00181536,

We thank you for the valuable comments.

1. We duly acknowledge the latest criteria for diagnosis of type 2 diabetes especially incorporating Ha1c level. Despite the addition of Hba1c into recent guidelines the diagnostic criteria for type 2 diabetes based on fasting or random sugar level does not change. We did incorporate Hba1c level for analysis among the two distinct groups in our study as suggested without affecting the study outcomes.
2. Being an original IRB approved retrospective cohort study by design at University of Chicago and having no new IRB approval for longitudinal follow-up data, we sincerely regret to inform that we would be unable to provide any longitudinal data at this time.
3. We have incorporated an additional table showing background data of NASH and NAFLD as suggested.
4. As suggested we have updated table 1 with data on ALT, AST, AST/ALT ratio and NASH diagnostic markers such as Fib-4 index, APRI score. All units of data have been incorporated for accuracy.

4. Reviewed by 02451447

   Comments to Authors

   The authors compared the histopathologic features in NAFLD patients with or without T2DM. The manuscript is well written and the study is well designed. Comments: 1. The pathologic diagnosis of NAFLD in advanced fibrosis, especially cirrhosis is difficult sometimes, since the features such as ballooning, perisinusoidal fibrosis or inflammation often do not present. How the authors make a NAFLD (cirrhosis) diagnosis and give the scores in these patients without typical pathologic features. 2. Glycogenated nuclei of hepatocytes often indicate the condition such as diabetes/insulin resistance. Are there any difference between patients with NAFLD with or without T2DM. I would like to see the comparison between these 2 groups. 3. The authors showed low platelet in advanced fibrosis patients with NAFLD with T2DM comparing that without T2DM. What is the explanation of this finding?

   Reply

   Dear Reviewer 02451447,

   We thank you for the valuable comments.

   1. Since this study was a retrospective review of biopsy proven NAFLD as determined by a well-trained histopathologist, the diagnosis of NASH was established by utilizing the NAS scoring system which is very objective.
   2. Glycogenated nuclei being not part of the NAS scoring system, was therefore not looked into as part of this study analysis.

3. Low platelets are well known to be associated with likely advanced fibrosis or cirrhosis, thus it is not surprising to see low platelet among patients with NAFLD and T2DM who also had much advanced fibrosis.

5. Reviewed by 02462691

Comments To Authors

This is a well-written piece although not novel. However, authors did try to contrast their studies from others. Any data for HbA1c? This may give an idea about diabetic control for the past months, and whether this can be a factor for more fibrosis? Likewise, treatment of diabetes may be a confounding factor since patients who were treated and well-controlled may have less severe disease. Although a single pathologist is ideal but since ballooning is such ill-defined form of finding, would there variation if the pathologist were to read the same biopsy one or two weeks later?

Reply

Dear Reviewer 02462691

We thank you for the valuable comments.

1. We have included HbA1c data for both groups respectively. The diabetic group had higher mean A1c essentially in uncontrolled range which downsizes the influence of diabetic medications and essentially proves that uncontrolled diabetes has a definite role in NAFLD progression.

2. Intraobserver variability of histological features of NAFLD if at all present is quite negligible as opposed to inter observer variability and would not change the overall outcomes. More studies are needed to explore the clinical significance of ballooning and its implications though.