Peer Review File

Article information: http://dx.doi.org/10.21037/dmr-20-75.

Reviewer A

The manuscript entitled 'Hepatic Steatosis Contributes to Fibrosis in Patients with Chronic Hepatitis B Infection'researched the relationship between these two causes of liver disease. Consecutive patients who underwent transient elastography were studied. Baseline laboratory and clinical data were collected. Univariate analysis and two-sided t-tests were performed, chi-square test for proportions, and logistic regression. The results show that patients with hepatitis B should be screened for fatty liver and the metabolic features. Liver enzyme elevation may occur independent of viral load replication. Fatty liver is associated with worsening degree of fibrosis in patients with hepatitis B. This research has certain clinical value.

Comment 1: Besides transient elastography and pathological grade manufacture specification, what other methods are currently used to evaluate liver fibrosis?
Reply 1: Agreed, other modalities for the evaluation of liver fibrosis should incorporated.
Changes in the text: We have modified the text as advised (see Page 5, lines 112-116; Page 8, lines 219-222)

Comment 2: This study has a lot of implications, and one of the suggestions from the research results is to screen for fatty liver in patients with hepatitis B. What kind of method is used to screen for fatty liver in patients with hepatitis B?
Reply 2: We suggest screening for fatty liver using a combination of laboratory and imaging assessments, we used the Fib-4 and TE with the CAP.
Changes in the text: We have modified the text as advised (see Pages 8-9, lines 219-229)

Comment 3: A very controversial issue with hepatitis B is fatty liver. Studies have shown that fatty liver can lead to more fibrosis or have no effect on hepatitis B patients. What is the underlying mechanism of this phenomenon?

Reply 3: We were not able to define a specific mechanism; however, suggested theory includes the etiology of steatosis.

Changes in the text: We have modified the text as advised (see Page 8, lines 209-218)

Comment 4: The increase of liver enzymes may occur independently of viral load replication, which may need to be considered when deciding to start antiviral therapy. For such patients, how to choose the timing of antiviral therapy?

Reply 4: This is an excellent question, which we hope our manuscript would incite further investigation.

Changes in the text: We have modified the text as advised (see Page 9, lines 236-238, lines 242-244)

Comment 5: Patients with steatosis and hepatitis B are more likely to have advanced fibrosis. Can the degree of hepatic steatosis be used as an indicator of prognosis in patients with hepatitis B?

Reply 5: No, we do not have the data of a longitudinal study to suggest the degree of hepatic steatosis can be used as an indicator of prognosis.

Changes in the text: We have modified the text as advised (see Page 9, lines 235-237)

Comment 6: In the case of overweight and obesity, we found that the BMI of more than 30 individuals in this cohort is lower than expected, and even in the case of obesity, there are more than 25 individuals in this cohort. Does this happen only in Asian populations? What strategies should be used to treat steatosis in patients with BMI below 25?

Reply 6: Disparities research does note that this finding is most common among Asian population. Currently, there is no specific recommendation for treatment with a BMI <25.

Changes in the text: We have modified the text as advised (see Page 8, lines 204-208)

Reviewer B

Comment 1: Page 2, line 49: word 9 “the” consider deletion

Reply 1: Agreed.

Changes in the text: We have modified the text as advised replaced with, “, of which,”

Comment 2: Page 2, line 56: "viral load replication" is a tautology. Consider expressing as either viral load, or viral replication.

Reply 2: Agreed.

Changes in the text: We have modified the text as advised, the word "load" was removed.

Comment 3: Page 3, line 63: need to define DNA and ALT

Reply 3: Agreed.

Changes in the text: We have modified the text as advised and definitions were provided.

Comment 4: Page 3, line 64: should be "is effective" not "has effective"

Reply 4: Agreed.

Changes in the text: We have modified the text as advised and the word "is" was replaced

Comment 5: Page 3, line 70: I question the wisdom of describing the most common risk factor for HCC in patients with medicare. I assume this is USA specific? Either define it as such (all Australians, for example, are covered by national Medicare) or delete.

Reply 5: We see your point and therefore have specified the Nationality with addition of the motive for the statement.

Changes in the text: We have modified the text as advised (see Page 4, lines 87-89)

Comment 6: Page 3, line 71: not true. PPAR-gamma agonists have trial data that shows benefit in NAFLD. Their use is restricted by side effects not efficacy (Ratziu V, Giral P, Jacqueminet S, Charlotte F, Hartemann-Heurtier A, Serfaty L, Podevin P, Lacorte JM, Bernhardt C, Bruckert E, et al. Rosiglitazone for nonalcoholic steatohepatitis: one-year results of the randomized placebo-controlled Fatty Liver Improvement with Rosiglitazone Therapy (FLIRT) Trial. Gastroenterology. 2008;135:100–110)

GLP-1 agonists also appear to be effective: Carbone LJ, Angus PW, Yeomans ND. Incretin-based therapies for the treatment of non-alcoholic fatty liver disease: A systematic review and meta-analysis. J Gastroenterol Hepatol. 2016 Jan;31(1):23-31. doi: 10.1111/jgh.13026. PMID: 26111358.

Not to mention the numerous other agents that are currently being trialled (obetacholic acid, coffee intake, etc.)

Reply 6: Thank you for the correction, we have made adjustments to this "absolute statement" and replaced it with "most cost effect." In addition, we provided citations for the literature you have provided.

Changes in the text: We have modified the text as advised (see Page 4, lines 91-95)

Comment 7: All patients were of asian background

Asian patients develop fatty liver more readily and at lower BMI than caucasians.

Mohanty SR, Troy TN, Huo D, O'Brien BL, Jensen DM, Hart J. Influence of ethnicity on histological differences in non-alcoholic fatty liver disease. J Hepatol. 2009 Apr;50(4):797-804. doi: 10.1016/j.jhep.2008.11.017. Epub 2009 Jan 1. PMID: 19231016.

Bambha K, Belt P, Abraham M, Wilson LA, Pabst M, Ferrell L, Unalp-Arida A, Bass N; Nonalcoholic Steatohepatitis Clinical Research Network Research Group. Ethnicity and nonalcoholic fatty liver disease. Hepatology. 2012 Mar;55(3):769-80. doi: 10.1002/hep.24726. PMID: 21987488; PMCID: PMC3278533.

Reply 7: Yes, these disparities references were included in our discussion.

Changes in the text: We have modified the text as advised (see Page 8, lines 204-208)

Comment 8: Page 5 line 138: was this statistically significant? P value?

Was there any association between use of antivirals and presence of fatty liver?

I note that all your averages are expressed as mean+/- SD. Are all the data normally distributed? I would be surprised if average ALT and duration of treatment were normally distributed. If they are not then average should be described as median+/- IQR.

A 2 sided t test can only be used on parametric data. I doubt all the data is parametric, if the data is indeed non-parametric then you need other tests such as wilcoxon rank.

Reply 8: For HBeAg levels, P-value was not statistically significant, p-value = 0.264. After running the Kolmogorov-Smirnov test, $\lambda = 0.119$, showing that there was moderate association between use of antivirals and presence of fatty liver. We ran the Kolmogorov-Smirnov test and used histograms to test for normality. We have modified the variables that do not follow a normal distribution by describing the average as median +/- IQR and analyzed them with the Mann

Whitney U Test.

Changes in the text: We have modified the text as advised (See Page 2, line 48; Page 6, lines 156-158; Page 7, lines 180-181, 185-186; Page 14, lines 368,375-376)

Comment 9: There are many studies that have looked at the interaction between fatty liver and CHB:

Zhu L, Jiang J, Zhai X, et al. Hepatitis B virus infection and risk of non-alcoholic fatty liver disease: A population-based cohort study. Liver Int. 2019;39(1):70-80.

Cheng YL, Wang YJ, Kao WY, et al. Inverse association between hepatitis B virus infection and fatty liver disease: a large-scale study in populations seeking for check-up. PLoS One. 2013;8(8):e72049.

Li WC, Lee YY, Chen IC, Sun C, Chiu FH, Chuang CH. Association between the hepatitis B and C viruses and metabolic diseases in patients stratified by age. Liver Int. 2013;33(8):1194-1202.

Worland, T., Apostolov, R., Asadi, K., & Leung, C. (2019). Hepatitis B virus activity is not associated with degree of liver steatosis in patients with hepatitis B-related chronic liver disease. Liver International, 40.

The aforementioned article by Cheng and colleagues also described an increased incidence of fatty liver in those with CHB, however most other work describes fatty liver at occurring at a similar rate in patients with CHB compared to background population.

The main limitation of most of these studies was definition for fatty liver, which was variably diagnosed on the basis of negative serology/history for other causes and ultrasound imaging suggesting the presence of steatosis. Only the Worland article used a more robust standard of fatty liver diagnosis in liver histology, and they found no association between activity of hepatitis B and presence/degree of steatosis.

May I suggest to add some of these aforementioned references to your manuscript and highlight what your research adds to the known literature.

Reply 9: After review of the references you provided, we completely agree. The above references were included into our manuscript.

Changes in the text: We have modified the text as advised (see Page 8, lines 209-218)

Comment 10: Your first paragraph of discussion (lines 158-162) describe "important findings," however none of these are unique and have all been shown in more eloquently designed and larger studies. From my reading your research is unique in that it uses CAP measurements in fibroscan to assess level of steatosis in hepatitis B. This is something that should be highlighted more in the article and pushed as a point of difference. How does your rate of fatty liver by CAP (about 60%) compare to rates seen in the population studies I mentioned above? This discussion needs significant re-writing to change this as the highlight. Your rate of fatty liver in CHB is much higher compared to that reported by Zhu et al (above), is this because you were examining asian patients living in the west compared to asia? Is it due to greater pick-up by using fibroscan? Both these points should be discussed. This would allow you to make a stronger case for publication as you could say from your results that by utilising better diagnostic methods that fatty liver occurs more commonly in CHB patients than previously reported.

Reply 10: Yes, the use of CAP was incorporated more heavily throughout the manuscript as a point of focus and the discussion was heavily edited to reflect our focus on health disparities among this cohort with the aforementioned emphasis. We believe these edits have significantly strengthened our case for publication.

Changes in the text: We have modified the text as advised (see Page 2, lines 38-63)

Comment 11: Page 6, line 167: If you want to speculate that CHB is a risk factor for progression of steatosis you need to tease out if increased viral activity is associated with a higher prevalence of fatty liver in your cohort. Was fatty liver as per CAP more common in those who were eAg pos, those with high HBV VL? The manuscript from Worland (above) looked at liver histology and thought not. This should be addressed.

Reply 11: Yes, given the provided citation, we have retracted our speculation of CHB serving as a risk factor.

Changes in the text: We have modified the text as advised (see Page 7-8, lines 187-218)

Comment 12: Page 7 line 180: should be "there have" not "there has"

Reply 12: Agreed.

Changes in the text: We have modified the text as advised and changes have been made to "there has"

Comment 13: Page 7 line 187: need to define MRE

Reply 13: Agreed.

Changes in the text: We have modified the text as advised and MRE was defined within our article.

Comment 14: Page 7 line 199: Again, you should concentrate on the unique aspect of your study, not on the stuff that is already well known. Fatty liver when determined by CAP occurs more frequently in patients with CHB than previously thought.

Reply 14: Agreed. The discussion section as mentioned was edited significant to focus on the uniqueness of our article.

Changes in the text: We have modified the text as advised (see Page 9, lines 240-247)
