Reply to reviewer

Dear Editor in Chief,

Thank you for sending me the respect reviewer's comments. I accept all of them and revised the manuscript according to their valuable recommendations. I respond to their questions point by point as the followings:

Comment 1: Obesity or adiposity may be a confounding factor for this association. As the authors wrote in Introduction, all the serum biomarkers are related with obesity. It may also be true that obese patients with NASH have elevated adiposity-related markers. Therefore, controlling for obesity index should be required to explain the association between NASH and biomarkers.

Reply: We do agree with the reviewer that obesity is a confounding factor when evaluating the association of adipokines and liver histologic findings. Indeed, the clinical variables including the BMI and waist circumference were entered in binary logistic regression analysis. Therefore the effect of BMI (obesity) was measured and controlled in the mentioned analysis. We corrected the sentence in the method part (statistical analysis) as the following:

"Binary logistic regression analysis using standard model was applied to evaluate the association of independent variables (including serum adipokines and clinical data) and liver histology findings."

Comment 2: The authors inconsistently suggested the cut-off for NASH components such as steatosis, inflammation, and fibrosis. Generally, binary variable can have only two values (yes or no). These variables have multiple kinds of values. To divide these variables into binary values, the reason should be suggested. With regard to fibrosis, periportal fibrosis as well as perisinusoidal fibrosis has only 1 point according to Kleiner et al. (2005). Kleiner et al. gave 2 points for perisinusoidal and portal/periportal fibrosis. How the authors categorize the group with both perisinusoidal and portal/periportal fibrosis?

Reply: We do agree with the reviewers comments about the ambiguity in regrouping the liver histology data in our binary logistic regression analysis. According to the aim of study, we wanted to show the association of independent clinical and laboratory data with the severity of steatosis, lobular inflammation, and fibrosis. Therefore we had to select the reference values for binary logistic regression analysis. We clarified the issue in the method part (statistical analysis) as the followings:

Hepatic steatosis severity was categorized into 4 degrees according to NAS. The first two degrees (0-1) represented for none/mild liver steatosis and the next degrees (2-3) showed moderate to severe amount of liver steatosis. To define the risk of lower liver steatosis versus more advanced degrees of steatosis, we considered the patients with steatosis grade of less than 33% as the "mild group". Meanwhile, those with higher degrees (2-3) were merged to form the "moderate to severe group".

Lobular inflammation range was graded from 0 to 3 in NAS. To estimate the risk of lower lobular inflammation against more advanced grades, we labeled the individuals with lobular inflammation of

less than two foci per HPF (grade 1) as the “mild group”. At the same time, those with higher lobular inflammation grades (2-3) were combined to form the “moderate to severe group”.

Hepatic fibrosis content was categorized into 5 stages based on NAS. The former two stages (0-1) demonstrated none/mild fibrosis and the latter stages stand for more advanced fibrosis (2-4). In order to determine the probability of lower fibrosis versus more advanced fibrosis, we labeled the subjects with perisinusoidal or periportal (stage 1) as the “mild group”. Those with higher fibrosis stages (2-4) were mixed to form the “moderate to severe group”.

For the regression model, liver steatosis, lobular inflammation, fibrosis stage, and NAS were employed as dependent variables; Steatosis grade of less than 33%, lobular inflammation of less than two foci per HPF, fibrosis stage of one (perisinosuidal or periportal), and NAS of five or higher were set as the reference groups, respectively.

Comment 3: How the authors categorize the group with NAS between 3 and 4? Did the subjects with simple fatty liver in Table 1 have NAS≤2 or NAS<5? Please clarify.

Reply: The subjects with simple fatty liver in table one had NAS≤2 and NASH group had NAS≥5. We defined the simple fatty group and NASH group according to the definition provided in the method part of study (the last two lines in “liver histology” section). To clarify the issue we added the following statement in the footnote of table one:

“Patients with non-alcoholic activity score (NAS) of five or higher were considered to have non-alcoholic steatohepatitis. Those with NAS equal to two or lower were defined as simple fatty liver[14].”

It worth mentioning that we reported the data of all participants, including those with NAS between 3 and 4, in the first column of table one. We preferred to report the data of “simple fatty liver group” and “NASH group” separately in the second and third columns of table one.

Comment 4: In Introduction, the authors described the need of non-invasive tool to diagnose with NAFLD. However, the authors did not suggest the reason why the adipokines may have role in NAFLD. Please enrich the contents in Introduction.

Reply: Thank you for your valuable comment. To explain the role of adipokines in NAFLD we added the following statement in the introduction part:

“NAFLD is a wide spectrum of liver cell injury that is induced by insulin resistance. Primary the accumulation of fat occurs in hepatoctyes (simple fatty liver) as a consequence of hepatic insulin resistance. A growing body of evidence supports that adipokines modulate these metabolic processes by regulating insulin mediated glucose metabolism, fatty acid utilization, and lipid accumulation of visceral tissues. At the later stages of disease, inflammatory phenomena arise that might progress to steatohepatitis and finally cirrhosis. It was suggested that the development of steatohepatitis could be a consequence of the balance between pro and anti inflammatory effect of adipokines.”

Best,

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