Dear Editor:

I am very grateful to reviewers' and your comments for the manuscript. According to your advice, we amended the relevant parts in the manuscript. Some of your questions were answered below.

Responses to Reviewer 1

1. The authors should check the relevance of some references. e.g. "Approximately 20-30% of NAFLD patients develop NASH, which includes a 20% risk of further causing cirrhosis [2]". "However, the pathogenesis of NAFLD and its progression is a complex process, and the "multiple-hit" hypothesis proposed by Buzzetti et al. in 2016 suggested that simple steatosis and NASH not only exhibited different risk of progression but might also reflect different disease entities in terms of pathogenesis [4]. Multiple insults including insulin resistance, obesity and gut microbiota contribute to the development of steatosis and liver inflammation [4]." "This percentage was significantly higher than that of the general adult population in China (approximately 15%), which was reported in 2013 [18]". The authors should check again all the references one-by-one to find the mismatches and correct accordingly.

Responses: We have rechecked all the references and corrected accordingly using EndNote X9 in the revised manuscript.

2. An incorrect statement occurs in abstract that BMI and HOMA-IR of the cirrhotic patients were higher than those of the NAFLD patients. Firstly, P values were not significant (P 0.912 and 0.539 respectively), and secondly mean values of BMI was

27.9 in NAFLD group (higher than that of Cirrhosis group, 27.7 kg/m^2).

Responses: The incorrect statement in the abstract has been revised. The BMI and HOMA-IR parameters of the cirrhotic patients were 27.7 kg/m2 and 9.57, respectively, which were significantly higher than those of the patients without NAFLD (P = 0.011 and 0.044, respectively).

3. The authors should discuss about the sensitivity and specificity of ultrasound to diagnose NAFLD.

Responses: We added the discussion about the sensitivity and specificity of ultrasound to diagnose NAFLD in the Discussion section, as following 'The sensitivity of ultrasound has been reported to range from 53% to 100% and its specificity from 77% to 98%[1]. Higher diagnostic sensitivities and specificities are achieved during the evaluation of moderate to severe hepatic steatosis cases, whereas lower values are noted during all grades of hepatic steatosis[1].'

References

32 Esterson YB, Grimaldi GM. Radiologic Imaging in Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. Clin Liver Dis 2018; 22(1): 93-108 [PMID: 29128063 DOI: 10.1016/j.cld.2017.08.005]

4. How many of 7 cirrhotic patients were decompensated?

Responses: Three cirrhotic patients were decompensated, one of whom had upper gastrointestinal bleeding, another two had hypersplenism, ascites, esophageal and gastric varices, and hepatopulmonary syndrome. And the

above statement has been added to 'Clinical characteristics of studied subjects' in the results section.

5. The second paragraph in page 15 should be checked and written again because many false statements exist.

Responses: The second paragraph in page 15 has been checked and been rewritten.

a. "Approximately 20 -30% of NAFLD patients develop NASH and this carries a 20% risk of causing cirrhosis [2]. However, only seven patients in the present study were diagnosed with cirrhosis". Actually, the incidence of cirrhosis in the present study population (patients with hypopituitarism and NAFLD) is calculated 7 patients with cirrhosis out of 27 patients with NAFLD (7/27 = 28%), while in general NAFLD population the incidence of cirrhosis is around 3%. So, the proportion of cirrhotic patients is high, and an explanation should be given in discussion for this high prevalence of cirrhosis, considering also that cirrhotic patients in this study were very young [mean age 21 years, range 19-25.2 years]. What do we know about rapidly progressive NAFLD due to hypopituitarism? What do other studies describe about cirrhosis prevalence in patients with hypopituitarism?

Responses: The incidence of cirrhosis in the present study population (patients with hypopituitarism and NAFLD) was 28%, which was significantly higher than that in the general NAFLD population but similar to that reported in a longitudinal cohort study (29%)[10, 20]. Common etiologies of fatty liver disease in patients of our present study were ruled out,

including hepatitis B or C, alcoholic fatty liver disease, drug and inherited diseases. We found a high prevalence of cirrhosis in hypopituitary patients, and there was not much research on the mechanism of NAFLD in pituitary/hypothalamic dysfunction. Thus, prospective, multicenter, cohort study and animal experiments are required in the future to facilitate further understanding of the NAFLD pathophysiology in hypopituitary patients.

References

10 Adams LA, Feldstein A, Lindor KD, Angulo P. Nonalcoholic fatty liver disease among patients with hypothalamic and pituitary dysfunction. Hepatology 2004; 39(4): 909-914 [PMID: 15057893 DOI: 10.1002/hep.20140]

20 Kabbany MN, Conjeevaram Selvakumar PK, Watt K, Lopez R, Akras Z, Zein N, Carey W, Alkhouri N. Prevalence of Nonalcoholic Steatohepatitis-Associated Cirrhosis in the United States: An Analysis of National Health and Nutrition Examination Survey Data. The American journal of gastroenterology 2017; 112(4): 581-587 [PMID: 28195177 DOI: 10.1038/ajg.2017.5]

b. "Thus, only two of our patients underwent liver biopsy. Therefore, we could not distinguish simple steatosis and NASH from NAFLD, and the incidence of cirrhosis might be misleading". Liver biopsy is to distinguish steatosis from NASH.

Responses: The prevalence of cirrhosis in hypopituitary patients in our present study was similar to that of a study of Adams *et al.*, which reported that in 10 patients with hypothalamic and pituitary dysfunction, whose

NAFLD symptoms were confirmed by liver biopsy, six patients were cirrhotic (29% of total cohort), two exhibited NASH with fibrosis (10.5% of total cohort), and two presented with simple steatosis [10]. In the present study, two of our patients underwent liver biopsy indicating the lack of differential diagnosis, between simple steatosis and NASH from NAFLD. Consequently, the incidence of NASH and that of fibrosis in our cohort was not clear.

References:

10 Adams LA, Feldstein A, Lindor KD, Angulo P. Nonalcoholic fatty liver disease among patients with hypothalamic and pituitary dysfunction. Hepatology 2004; 39(4): 909-914 [PMID: 15057893 DOI: 10.1002/hep.20140]

c. Epistaxis is not a manifestation of cirrhotic decompensation. Did delay in diagnosis and treatment initiation contribute to progression to cirrhosis? An increased HOMA-IR was found to cirrhotic patients and this finding was presented as a potential factor that lead to progression to cirrhosis. However, as we know liver cirrhosis and particularly portal hypertension could lead to insulin resistance and this probability should be discussed.

Responses: In the study of Adams *et al.*, mean age of the 21 patients with NAFLD and pituitary/hypothalamic dysfunction at time of diagnosis of NAFLD was 36 ± 22 years (range 9-78), who were diagnosed with NAFLD 6.4 ± 7.5 years (median 3 years) after the diagnosis of pituitary/hypothalamic dysfunction. And the 7 cirrhotic patients in our present study were diagnosed with cirrhosis 13.0 (11.0-18.0) years after the diagnosis of

hypopituitarism. There was no evidence confirming that delay in diagnosis and treatment initiation contributed to progression to cirrhosis. In this retrospective study, we found that HOMA-IR in patients with hypopituitarism and cirrhosis was significantly higher than that in patients without NAFLD. And fasting insulin concentration was positively associated with plasma osmolality in patients with NAFLD, although no correlation was noted between HOMA-IR and plasma osmolality. However peripheral IR and pancreatic β-cell dysfunction even diabetes mellitus may occur in cirrhotic patients. Thus, the effect of the hyperosmolar state on the progression of NAFLD in hypopituitary patient needs further studies.

6. Plasma osmolality and sodium concentration was statistically significant higher in cirrhotic patients when compared only to NAFLD group. Why wasn't this difference found between cirrhotic and NAFLD (-) patients?

Responses: There may be some reasons. Firstly, the number of cirrhotic cases was small. Secondly, a longitudinal cohort study may be required, and the area under the blood sodium and blood osmotic pressure curve should be calculated, which may be better reflect the real situation of patients.

Response to Reviewer 2

1. In the section "Association with metabolic syndrome" there is an incorrect statement that "the median HOMA-IR of the cirrhotic, the NAFLD (+) and the NAFLD (-) groups was 9.57, 3.92 and 2.60, respectively": there should be "the NAFLD group" (as

indicated in Table 1) or "the NAFLD patients". I also recommend removing from the abstract: "The parameters BMI and HOMA-IR of the cirrhotic patients were … higher than those of the NAFLD patients" as incorrect and/or not significant. "The plasma osmolality and serum sodium levels of hypopituitary patients with cirrhosis were higher than subjects with NAFLD and those without NAFLD" is a false statement, because levels were "significantly higher than those of the NAFLD patients".

Responses: We have revised the incorrect statement in the section "Association with metabolic syndrome" and the Abstract.

2. The statement, "that hyperosmolality might be a contributor to the deterioration of NAFLD in hypopituitary patients" cannot be considered valid only on the basis that "the plasma osmolality and serum sodium concentration of the cirrhotic patients were … were significantly higher than those of the NAFLD patients", because there were no differences with patients without NAFLD. In addition, since it is known that hyponatremia, but not hypernatremia, is a frequent feature of hypopituitarism, this should be discussed in relation to NAFLD.

Responses: Hyponatremia is a frequent feature of hypopituitarism, however, 72% of patients with hypopituitarism presented with central diabetes insipidus. Thus, hypernatremia was manifested in these hypopituitary patients. In this retrospective study, we found that the plasma osmolality and serum sodium concentration of the cirrhotic patients were significantly higher than those of the NAFLD patients. There was not much research on the mechanism of NAFLD in pituitary/hypothalamic dysfunction, and the

statement "that hyperosmolality might be a contributor to the deterioration of NAFLD in hypopituitary patients" was not valid. Thus, prospective, multicenter, cohort study and animal experiments are required in the future to facilitate further understanding of the NAFLD pathophysiology in hypopituitary patients.

3. The text states "that there were no significant differences in ... diastolic blood pressure (DBP) between NAFLD (+), and NAFLD (-), patients (P = 0.050), respectively", but the Table 1 shows a significant difference.

Responses: We rechecked the statistic results, and the text states in the section "Association with metabolic syndrome" was right, and we have revised the incorrect in the Table 1.

4. Statement "that the hyperosmolar state may aggravate NAFLD in hypopituitary patients by exacerbating insulin resistance" needs to be confirmed, since it is well known that NAFLD is intimately related to insulin resistance, and the osmolality was statistically significant higher in cirrhotic patients when compared only to NAFLD patients in the study. The correlation between the HOMA-IR and the osmolality should be calculated.

Responses: A correlation analysis was done, fasting insulin concentration was positively associated with plasma osmolality in patients with NAFLD, after adjusting for gender, age, and BMI (*r* 0.540, *P* 0.046), but no correlation was noted in total hypopituitary patients or in patients without NAFLD. There was no correlation between HOMA-IR and plasma osmolality. There

may be some reasons for no differences in plasma osmolality and sodium concentration between cirrhotic and NAFLD (-) patients. Firstly, the number of cirrhotic cases was small. Secondly, a longitudinal cohort study is required, and the area under the blood sodium and blood osmotic pressure curve should be calculated, which may be better reflect the real situation of patients. And animal researches are required to confirm this conclusion.

5. The article lacks the latest references (2017-2018), and all available references should be carefully checked for relevance.

Responses: We have appended the latest references (2017-2018) and rechecked all available references.

Responses to Editor

1. The revised manuscript has been edited by the American Journal Experts (AJE), and the language certificate is submitted.

2. The figure resubmitted is editable.

3. In the results section, we removed the *P* values that were > 0.05.

4. We have added the article highlights in the revised manuscript.

For easy check, these revised parts in this version of the manuscript are highlighted in red. Many grammatical or typographical errors have also been revised.

Thank you and all the reviewers for the kind advice.

Sincerely yours,

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