

Dear Editors,

Ms: World Journal of Hepatology - 80434

Title: Liver test abnormalities in asymptomatic and mild COVID-19 patients and their association with viral shedding time

We sincerely thank the editors and reviewers for the helpful feedback. We have revised our manuscript according to the reviewers' comments, and the itemized response to each comment is attached.

Sincerely,

Dr. Xueqin Chen, on behalf of co-authors

Response to Reviewer 1:

1. Considering changing the title of the study to be more reflective of the research's goal.

Author response: Thanks for your suggestive comments. The goal of our research is to determine the clinical characteristics of liver test abnormalities in asymptomatic and mild COVID-19 patients and their association with the viral shedding time. Therefore, the title remains "Liver test abnormalities in asymptomatic and mild COVID-19 patients and their association with viral shedding time" after our careful consideration.

2. Shortening the results section in the study abstract and highlighting only the most important findings of the current study.

Author response: Thanks for your suggestion. We have shortened the result section in the abstract according to your comments.

3. The introduction to the study, as well as the paper material and methods section, are written in a distinct style, and the references on which it is based are recent.

Author response: Thanks for your helpful suggestion. We have confirmed all the references are recent.

4. Examine the progress of the results in the paper again to avoid any incoming errors that could jeopardize the research's credibility.

Author response: Thanks for your helpful advice and we have revised them.

5. Adding a paragraph indicating the possibility of re-verifying the study's results if any party wishes to confirm the results' credibility.

Author response: Thanks for your advice and we have revised it.

Methods section: “The results of statistical analyses can be re-verified if any party wishes to confirm the credibility.”

6. To avoid future reader confusion, avoid using subheadings in the results section.

Author response: Thanks for your kind suggestion. In fact, our results section are composed of three different subsections, namely “study design and participant criteria”, “clinical features of COVID-19 patients with liver test abnormalities” and “association between viral shedding time and liver test abnormalities”. Each subsection includes a large quantity of contents, and the subheadings can make the contents organized and comprehensible. Therefore, we remain the subheadings in the results section after our careful consideration.

7. Separate the conclusion from the discussion section and expand the discussion section.

Author response: Thanks for your suggestion and we have revised them.

8. Please reformulate the study's conclusion.

Author response: Thanks for your suggestion and we have revised it.

9. Replacing reference number 22 with a more recent reference from 2020 and beyond.

Author response: Thanks for your suggestion and we have revised it.

“22 Jacobsen, Henning, and Sabra L. Klein. "Sex differences in immunity to viral infections." *Frontiers in immunology*. 2021: 3483. [DOI:10.3389/fimmu.2021.720952]”

10. Check the plagiarism and self-citation percentages of references to ensure they are within the accepted journal policy.

Author response: Thanks for your suggestion and we have checked them.

Response to Reviewer 2:

1. Introduction: “In addition to respiratory symptoms and fever, 14-69% of patients

with COVID-19 have abnormal liver function tests, mainly manifested by transient elevations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST)” A review article on the same topic observed that the most common liver function abnormalities are hypoalbuminemia and followed by elevation of gamma-glutamyl transferase. [doi: 10.4254/wjh.v13.i5.522] Please correct this discrepancy.

Author response: Thanks for your suggestive comments. We appologize for the mistake and have revised it.

“In addition to respiratory symptoms and fever, 14-69% of patients with COVID-19 have abnormal liver function tests, mainly manifested by elevations of hypoalbuminemia, gamma-glutamyl transferase, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [2,3].”

[2] Nasa, Prashant, and George Alexander. "COVID-19 and the liver: What do we know so far?." World Journal of Hepatology 2021;13(5): 522.

2. Measures: “Liver diseases included chronic hepatitis B and alcoholic/nonalcoholic fatty liver disease (NAFLD).” This statement is incomplete. Please rephrase this statement.

Author response: Thanks for your suggestion. We have modified the statement in the manuscript.

“Liver diseases included chronic hepatitis B, alcoholic/nonalcoholic fatty liver disease (NAFLD) and other liver diseases.”

3. Results: A total of 130 (19.7%) had underlying diseases, of whom 57 (8.6%) had liver diseases. 45 (6.8%) had NAFLD, 11 (1.7%) had hepatitis B, and 1 (0.2%) had both. The number of patients with elevations in ALT, AST and TBIL was 53 (8.0%), 61 (9.2%), and 4 (0.6%), respectively, with a majority of mild liver test abnormalities. How many of these patients were preexisting liver test abnormalities? Was the elevation in the patients with preexisting abnormalities was 3X from the base line or 3X from ULN?

Author response: Thanks for your question. Actually, we failed to obtain the preexisting liver test results of the COVID-19 patients. The aim of our study was to determine the clinical characteristics of liver test abnormalities in asymptomatic and mild COVID-19 patients. Therefore, we focused on the liver test results during the onset of the disease rather than the preexisting abnormalities.

4. A multivariate logistic regression model for key factors indicated that liver test abnormalities were only associated with a history of liver disease (OR 8.004, 95% CI

4.319-14.835, $P < 0.001$). A multivariate logistic regression model for all factors showed that liver test abnormalities were significantly associated with the age of 30-49 years (compared with age 14-30 years, OR 1.970, 95% CI 1.073-3.618, $P = 0.029$), male sex (OR 1.728, 95% CI 1.005-2.971, $P = 0.048$), and a history of liver disease (OR 8.265, 95% CI 4.315-15.831, $P < 0.001$). How two different set of factors were significantly associated with liver test abnormalities in multivariate analysis?

Author response: Thanks for your comments. In fact, we performed two multivariate logistic regression models to identify risk factors associated with liver test abnormalities. The two models could help to establish the conclusion more firmly. All factors included age, gender, body mass index, comorbidities, disease type, symptoms and medication. Key factors were the correlated factors in the univariate model. According to the statistical results, the multivariate logistic regression model for key factors indicated that liver test abnormalities were only associated with a history of liver disease. The multivariate logistic regression model for all factors showed that liver test abnormalities were significantly associated with the age of 30-49 years, male sex, and a history of liver disease.

5. Two types of multivariate linear regression were performed, with key factors and all factors. What are 'key factors' and 'all factors'?

Author response: Thanks for your question. All factors were the literal meaning of all the factors including age, gender, body mass index, comorbidities, disease type, symptoms and medication. Key factors were the correlated factors in the univariate model. We have mentioned the definitions in the results section- *Clinical features of COVID-19 patients with liver test abnormalities*.

6. What was the criteria used for viral shedding time? Positivity of RT-PCR and CT value in RT-PCR

Author response: Thanks for your question. In our research, viral shedding was defined as the cycle threshold (Ct) values of both ORF1ab and N greater than 35. Ct values below 35 were considered positive for COVID-19 infection. We have mentioned the definition in the methods section- *Measures*.

7. It was mentioned that all cases of Omicron BA2.2 were recruited in the study. How the variation was identified, through genotyping or any other method ?

Author response: Thanks for your kind comments. The Omicron BA2.2 variation was

identified through genomic sequencing. According to a previous research[12], the phylogenetic features of SARS-CoV-2 viral genomes from 129 patients in this period, and inferring their relationship with those available on the GISAID database, indicated that all of the viral genomes were clustered into the SARS-CoV-2 BA.2.2 sub-lineage. Since all cases of our study were from the same period and location as the above study, we consider they were infected with Omicron BA2.2.

8. Discussion: One possible reason for this was that our participants were all asymptomatic carriers or mild cases, among whom abnormal liver test results were more rare. Change more rare to uncommon.

Author response: Thanks for your suggestion and we have revised it.