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OPEN PEER REVIEW

Relationship Between Serum HBV-RNA Levels, Sero-biochemical Indices, and Clinicopathological Parameters in Patients with Hepatitis B Virus

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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Contents

1 **Original submission** Referee 1, Associate Editor 2
2 **Revision 1** Nazila Bahmaie, Referee 1, Associate Editor, Editor-in-Chief 3
Authors' reply to reviewers 4
Reviewer and editor comments 11

1 Original submission

2 comments · 26 Feb 2024 – 1 Mar 2024

Referee 1 Reviewer

MINOR REVISION 26 Feb 2024

Dear Ae,
The study titled "Relationship between serum HBV-RNA levels and biochemical parameters, serological indexes, and clinicopathological parameters in patients with hepatitis B virus infection-related diseases" investigates the correlation between serum HBV RNA levels and various clinical parameters in patients with different stages of chronic HBV infection.
Here are some potential flaws that need to be addressed in the study:
-Some sentences lack references. All sentences quoted from other articles must have references at the end of each sentence. For example:
HBV infection can lead to chronic hepatitis B, hepatitis B cirrhosis, hepatocellular carcinoma, and other related diseases. HBV infection is a public health problem worldwide.

The symptoms of early HBV infection are difficult to detect. Liver biopsy is still considered the gold standard for the diagnosis of liver cirrhosis.
- The manuscript needs an English edit.
-Please explain in detail in the methodology the settings and locations where the data were collected.
-Please add a participant flow diagram to your study.
-You reported that serum from 155 patients was used. How did you determine that a certain minimum number of research participants was required?
-While the study identifies correlations between serum HBV RNA levels and methionine metabolism, it does not delve deeply into the underlying mechanistic pathways or biological mechanisms driving these associations. Exploring the mechanistic insights behind the observed correlations could enhance our understanding of the pathophysiology of HBV infection and identify potential therapeutic targets.

Associate Editor

MAJOR REVISION 1 Mar 2024

Dear EIC,
Thanks to the authors and appreciation to the researchers. The manuscript presents valuable research. But some revisions are needed. The authors should revise it based on the reviewers' comments. Also, a minor change should be made in the abstract:
The study design should be mentioned in the first line of the methods section of the ABSTRACT.

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor and Reviewers,

Thanks for providing your suggestion on our manuscript. We revised the manuscript based on your suggestions and provided our responses to each comment below.

Reviewers' Comments:
Thank you for your comments. We have made changes in the manuscript according to your suggestions. We have also added new information regarding the study and interpretation of our data.

Reply to Reviewer:
Reviewer 1:
Dear Editor,
1. The article does not mention the criteria for diagnosing diseases related to hepatitis B, additionally, there is no information about viral treatments or the duration of the disease.
Response: Thank you for your suggestions. Patients were diagnosed based on the criteria specified as per the diagnostic criteria for chronic hepatitis B of the Chinese Medical Association, 2015 edition. We added and cited this reference. Now, we added the information related to current antiviral treatment of the patients. We have not evaluated the impact of patient medication on the different parameters as some patient records lacked current or previous medication information before enrolling to the study

2. The article should mention the patients' consent.
Response: We have revised the material and methods part and added the procedure followed for obtaining patients consent. (Page 7; Line 21-22).
3. The methodology used in the study should be explained in more detail, including the type and volume of the sample for viral genomic extraction and its steps.
Response: We have revised the part following your suggestion. (Page 8; Line 8-15)

4. Information about the primers and probe used, the type of fluorochrome, and which virus gene was amplified should be included.
Response: We have added the information as mentioned in the manufacturer's website and protocol. All operations were carried out in strict accordance with the instructions protocot provided by the manufacturer as instruction. (Page 9; Line 6-13).

5 The standard used for the quantitative measurement of RNA should be specified.
Response: We have revised the part following your suggestion.

6. The tables need more explanation and should be included in the manuscript.

Response: Overall findings of each table is included in the result section, and further explanations and implications of these findings are included in the discussion section.

7. Furthermore, the article should explain the reason for the lack of correlation between RNA level and methionine in cases of cirrhosis, fibrosis, and asymptomatic patients.
Response: We have now addressed the lack of correlation between RNA level and methionine in cases of cirrhosis, fibrosis, and asymptomatic patients in the result section 3.3 as well as in the discussion section.

Reviewer 2:
Dear knowledgeable and resourceful authors
Greetings!

Thank you for your great interest to submit your manuscript in this journal.

1. The title of your study is good, BUT A BIT long and LESS comprehensive in a same time, WITH some ambiguity, it arising a barrage of questions. I would like to kindly and highly recommend you to summarize it like this: Relationship between Serum HBV-RNA Levels, Sero-biochemical Indexes, and Clinicopathological Parameters in Patients with Hepatitis B virus.
What is more, you should meet the criteria of the journal for number of the words used in the main title. Accordingly, after these changes that you are going to do in your main title, you should count the number of words. Afterward, try to develop and synchronize your running title appropriated to main title.
Response: Thank you for your suggestions. We have changed the title of the article.

2. Caution!!!!Please remember all comments for abstract should be considered as same as those for related parts in article. Because, totally, all parts of the abstract and main body of your manuscript should be synchronized in order to be comprehended easily by readers and weighed up precisely by reviewers.
Response: We have extensively revised the abstract of the article.

3. We highly recommend you to specify a brief description of your biostatistics, version of software and considered p value for your results, right after the last sentences related to the material and methods.
Response: We have added the detailed information as requested in the materials and methods section, subsection ‘2.6 Statistical analysis’**.** Please refer to lines number 18.

4. It seems that your “conclusion” part is full of ambiguity and it has not been written by detail. Also, this is a repetition of title.
There is a prominent point that all of the basic medical scientists should be eligible to do. They are always finding a response through basic sciences in order to assist clinicians, eventually. Hence, a clinical outcome or change in clinical manifestations, and lab results should be mentioned in conclusion of the abstract and article resulted

from this immunomodulation.

One thing that is of high prominence in this part, is that it should be written according to authors conception with any recommendation or further investigations especially is desired not to be written by sheer or unconditional words/verbs. Because there is nothing absolute in medicine or medical basic sciences!!!!!
Response: The conclusion now elaborates on the following:

1. Brief summary of our findings
2. Key highlights of our study are indicative of the biological significance of our study.
3. Reasoning as to how our study provides a fresh and unique approach to studying chronic Hepatitis B infections in patients.
4. Future directions of this study based on the limitations.

5. It seems that your “conclusion” part is full of ambiguity and it has not been written by detail. Also, this is a repetition of title.
There is a prominent point that all of the basic medical scientists should be eligible to do. They are always finding a response through basic sciences in order to assist clinicians, eventually. Hence, a clinical outcome or change in clinical manifestations, and lab results should be mentioned in conclusion of the abstract and article resulted from this immunomodulation.

One thing that is of high prominence in this part, is that it should be written according to authors conception with any recommendation or further investigations especially is desired not to be written by sheer or unconditional words/verbs. Because there is nothing absolute in medicine or medical basic sciences!!!!! (CONCLUSION OF THE ABSTRACT).
Response: Thanks for suggestion, we revised the conclusion section both in the abstract and in the main manuscript text.

6. As I read the first and the second paragraph of your introduction part, I found out that it is well-written in comparison with your introduction part in the abstract. Accordingly, I highly recommend you to decipher the “cause and effect” especially molecular and immunopathophysiological aspect of the study. I mean it is better that you decipher them and increase the quality of the content and refrain from any ambiguity (INTRODUCTION PART).
Response: The abstract only has brief background information on the objective and introduction to our study. In the introduction section, the following has been addressed:

1. The molecular biology-based tests that are used to detect the diseases.
2. The use of HBV RNA instead of the HBV covalently closed circular DNA and this would explain why this molecule was also used in our study.
3. Evaluating the liver amino acid metabolism and how it is relevant to the liver diseases being examined in this study.

Based on these relevant aspects, serological analyses were performed in patients that satisfied both the “molecular and immunopathophysiological” aspects of this study.

7. I highly recommend you try to explain and present the SERO-immunopathophysiology of the disease and involved genes/FACTORS/INDEXES mentioned in this concept in a schematic presentation by Photoshop or PowerPoint in order to qualify and signify the concept and purpose of study (INTRODUCTION PART).
Response: Dear reviewer thanks for your suggestion. Figure 1 (new figure) in the manuscript provides a graphical representation of the introduction.

8. It is very of advantage that you show your authority by using your own references and data of last studies. From this aspect, I believe that it is not acceptable as cause and effect. The previous paragraph looks like Just a literature review. But I highly recommend you try to accredit purpose of your study and importance of investigation of these genes in case of cancer through depicting the reasons and level of successfulness of recent therapeutic approaches, more and more (INTRODUCTION PART).
Response: Dear reviewer thanks for your suggestion. Relevant and recent publications in the introduction section to provide detailed information on the current practices of diagnostic markers used in the field of liver diseases have now been included in the manuscript.

9. What is ethical code approved by ethical committee for this study? It should be inserted here, in the first paragraphs of material part. (METHODS PART).
Response: Dear reviewer thanks for your suggestion. We have addressed the missing information in ‘2. Methods’. We added the ethics committee approval number (20230728-53).

10. Was ALL OF THE DETAILS IN THE METHOD PART set up or gathered from previous researches? If so, please mention its reference(s). If it is set up by yourselves, please mention it and bear in mind presents your reasons for them. This comment should be considered for entire material methods part (other parts are no exception).
Response: Dear reviewer thanks for your suggestion. Wherever applicable, we have provided references for each technique used.

11. Be careful that it is “discussion” not “literature review”. Majority of authors falsify the discussion with literature review. Even the literature review that you did, is good and shows your authority in your article, but is not enough. Finally, due to not considering other researches, It will be considered as a negative point for your 11 of rearticle, clearly. In this part, you should study previous similar studies and discuss the identities, differences and their reasons and compare them with your acquired data. For instance, you should imply to other processes (especially with therapeutic strategies which are

based on immunological pathways), doses of injections, times and repeats of injections, routs of injections (in all cases if there is any injection), cultures, cells, stage or severity of the disease that animal lab were experimented, and drugs or agents and ….. (FOR DISCUSSION PART).

Response: The discussion section has now been modified heavily to include more studies that can be compared and discussed in the context of our study. We have also included explanations for the findings from our study in the context of the relevant studies published in recent times. We have also provided a potential mechanistic discussion that touches upon the molecular parameters that may influence the results we observe.

12. Please ascertain mentioned point by using “original or experimental article” that are published newly. Also, the name of corResponseing writer or first author should be clarified. Please write the name of researchers in italic form . (FOR DISCUSSION PART).

Response: Dear Reviewer, thanks for your suggestion; however, we already mentioned the other literature with their citation in the discussion sections without adding the initial author.

13. As one of the most important part for decisions made by readers and reviewers, even specialists, clinicians and technical managers of clinical diagnosis laboratories, you should be careful about “conclusion” part. It should not be a repetitive part of your abstract.

Please refer to the comment that is placed in the first page (abstract) and also refer to journal criteria and set your “conclusion” part according to journal instruction and my important points (FOR CONCLUSION PART).

Response: Dear reviewer thanks for your suggestion. Similar concern was stated in comment 4 ; this section has been reworded extensively so as to provide more accurate and pertinent inferences as well as implications.

14. Totally, you should re-write the conclusion part. (FOR CONCLUSION PART).

Response: Dear reviewer thanks for your suggestion. The same as for comment 13.

15. Please and please, investigate more articles for your study as well as updated ones in order to improve the quality of purpose behind your study (REFERENCE PART).

Response: Dear reviewer thanks for your suggestion. The reference list now includes more publications that are recent as well as relevant, that support our study/findings.

Reviewer 3:

Dear Ae,

The study titled "Relationship between serum HBV-RNA levels and biochemical parameters, serological indexes, and clinicopathological parameters in patients with hepatitis B virus infection-related diseases" investigates the correlation between serum

HBV RNA levels and various clinical parameters in patients with different stages of chronic HBV infection.
Here are some potential flaws which need to be revised in the study:
1. Some sentences lack references. All sentences quoted from other articles must have references at the end of themselves. FOR example:
HBV infection can lead to chronic hepatitis B, hepatitis B cirrhosis, hepatocellular carcinoma, and other related diseases. HBV infection is a public health problem worldwide.

Response: Dear reviewer, Thanks for your suggestion. We added a new reference for the following statement "HBV infection is a public health problem worldwide.":
World Health Organization Hepatitis B. 2021. [March 7, 2022]. https://www.who.int/news-room/fact-sheets/detail/hepatitis-b

2. The symptoms of early HBV infection are difficult to detect. Liver biopsy is still considered the gold standard for the diagnosis of liver cirrhosis.
Response: Dear reviewer, Thanks for your suggestion. We added a new reference for the above statement.
Attallah, K., et al., HCC-Check: A Novel Diagnostic Tool for Early Detection of Hepatocellular Carcinoma Based on Cytokeratin-1 and Epithelial Membrane Antigen: A Cross-Sectional Study. Technology in cancer research & treatment, 2024. 23: p. 15330338241234790.

3. The manuscript needs an English edit.
Response: The manuscript has now been improved in language, grammar, and accuracy.

4. Please explain the settings and locations where the data were collected, in the methodology in detail.
Response: Dear reviewer thanks for your suggestion. This information is now addressed in section '2. Methods'.

5. Please add a participants flow diagram to your study.
Response: Dear reviewer thanks for your suggestion. The flow diagram has been added as Figure 2.

6. You reported that serum of 155 patients were used. How did you determine that a certain minimum number of research participants was required?
Response: A total of 155 participants were included in the study due to the short duration of our study duration (9 months), and we found very limited number of patients in our hospital (center) as per our inclusion criteria. We agree that a large study would be required and recommend a study with large sample size.

7. While the study identifies correlations between serum HBV RNA levels and methionine metabolism, it does not delve deeply into the underlying mechanistic pathways or biological mechanisms driving these associations. Exploring the mechanistic insights behind the observed correlations could enhance our understanding of the pathophysiology of HBV infection and identify potential therapeutic targets.
Response: Dear reviewer thanks for your suggestion. The discussion section now includes an in-depth explanation/potential reasoning for the molecular mechanisms for these associations and how therapeutic strategies could be improved.

Associate Editor's Comments:

Associate Editor 1:
Dear EIC,
Thanks to the authors and appreciation to the researchers. The manuscript is a valuable research. But some revisions are needed. Authors should revise it based on reviewers comments. Also a minor change should be done in abstract:
Study design should be mentioned in first line of method section of ABSTRACT
Response: The method section now focusses on the study design in the first line rather than the objective of the study.

EIC Decision:

Major revision

2 Revision 1

4 comments · 10 May 2024 – 12 May 2024

Nazila Bahmaie Reviewer — ACCEPT 10 May 2024

Thank you for revising the manuscript. You succeeded in achieving good academic writing and a logical presentation of the data.

Referee 1 Reviewer — ACCEPT 12 May 2024

Dear AE,
I reviewed the manuscript again. The great effort in improving the manuscript is highly appreciated.
The authors have addressed all comments.
Thank you

Associate Editor — ACCEPT 12 May 2024

Dear EIC.
Previous errors have been corrected, and there are no further recommendations.

Editor-in-Chief — ACCEPT 12 May 2024

Dear Authors,
Your revisions have significantly improved the article, and it is now deemed acceptable for publication.
Congratulations on your hard work and dedication to enhancing the quality of your work.