General comments of reviewer 1

Reviewer #1:

Scientific Quality: Grade C (Good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Minor revision
Specific Comments to Authors: In this manuscript, the author conducted a series of experiments to evaluate the serum concentration and proteolytic capacity of MMP-2, -7, and -9 in CHC patients, according to fibrosis progression. This paper is written smoothly with clear thinking, but there are some minor problems

We sincerely appreciate all your observations, which undoubtedly will increase the quality of our paper. We send again the manuscript to a native speaker of English for the language evaluation

1. CHC patients were categorized in fibrosis grades through FibroTest and/or FibroScanASA. FibroTest and/or FibroScan ASA, it is best to attach a table to explain.
Answer:
The fibrosis stages of patients classified by FibroScan and FibroTest were grouped into similar intermediate classifications (F0-F1, F1-F2, F2-F3, and F3-F4). Patients whose tests were concordant or in close stages were included, whereas patients whose results were discrepant were discarded. That information, together with a supplementary table, was added to the Material and Methods section.

	F0	F0-F1	F1	F1-F2	F2	F2-F3	F3	F3-F4	F4
FibroTest® n	36		9		12	2	15	5	38
FibroScan® n	30	6	7	2	10	4	18	2	38
Total n FibroTest® / FibroScan®	36		11		14		20		38

2. In line 21 of page 5, “has” should be changed to “have”.
Answer: We changed “has” to “have”.

3.In line 20 on page 6, “suggest” should be changed to “suggested”.
Answer: We changed “suggest” to “suggested”.

4.In line 24 of page10, “area” should be changed to “areas”.
Answer: We corrected both the name of the curve and the verb, as follows: …the area under the receiver operating characteristic (AUROC) curve for MMP-7 was determined…

5. On page 12, “Mean patient age was between 55 and 60 years and the BMI was higher in F3”. May be wrong? From Table 2, F4 was higher.
Answer: The reviewer is correct. We made the change: “was higher in F4”.

6. On page 12, ROC values >0.7 were considered acceptable. The results showed that MMP-7 was not effective for distinguishing F0, F1, and F2 (Figure 4A). May be involved F3?
Answer: The reviewer is correct. The ROC values <0.7 were not significant. We made the correction: "The results showed that MMP-7 was not effective for distinguishing F0, F1, F2, and F3 (Figure 4A). However, the ROC values were acceptable in F4, the advanced fibrosis stage (Figure 4A, B). The use of MMP-7 as a complementary protein could improve the specificity and sensitivity of the available methods for determining that fibrosis stage."

7.In the final "discussion" part, the results obtained in Table 1 and Table 2 were not discussed. Overall, I think this article has certain innovation, but there are also some problems. I think this article needs to be reviewed again after revision.
Answer: We added the following information in the Discussion section**.**
The demographic data revealed that the CHC population was older than the control group, correlating with the progression and evolution time of hepatitis C, which is usually diagnosed in advanced-age patients. In contrast, the control individuals were young, which is the common age range of blood donors (Zucoloto et al. 2019). Furthermore, both the CHC and control subjects presented with the obesity criteria, with no apparent impact on biochemical values, including platelets and liver enzymes (AST, ALT, and GGT), which showed evident clinical alterations in CHC. Moreover, young patients were mainly identified as F1, which possibly was related to the fact that some of the study subjects then became blood donors (whose common age range is from 35 to 45 years), and through the viral panel, were found to be positive for the hepatitis C virus. Advanced stages of fibrosis were found in patients whose age ranged from 55 to 60 years.

Reviewer #2:

Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Accept (General priority)

Specific Comments to Authors: This work evaluated the role of MMPs in the pathophysiology of liver fibrosis in CHC patients. It was revealed that the regulation of MMP ́s can be served as possible therapeutic targets for improving novel strategies to reverse liver fibrosis in CHC. The experimental design is reasonable and the work is worthy of further study.

We greatly appreciate the valuable time taken by the reviewer to evaluate our manuscript, knowing that the comments and questions improve our paper's quality. Our point-by-point response to each of the comments and questions follows below.

There are only some small questions:

1. If the number of samples can be expanded, the evaluation of data may be more convincing.
Answer: As the reviewer states, a higher number of patients could improve the sensitivity and specificity of MMPs, mainly for MMP-7, as biomarkers for fibrosis stages. Importantly,

our research group is currently evaluating those and other proteins as serologic biomarkers. We consider that the present study is a first step, before designing a novel panel to differentiate fibrosis stages in chronic liver disease. From 2010 to 2019, studies that focus on the determination of MMP concentration in liver diseases include a modest number of patients (20 to 60 patients) (Helaly 2011; Irvine et al. 2016; Latronico et al. 2016; Prystupa et al. 2015; Thomas 2018). Thus, we consider that our study contributes valuable knowledge in the area of liver disease.

2. I am confused about the statistical analysis in the Figures, because usually we use the way of *$p<0.05$, ** $p<0.01$, *** $p<0.001$. Please check all the related results.
Answer: We unified the manner of describing the p values throughout the manuscript, in accordance with the reviewer's comments.

3. As was mentioned, the concentration and activity of MMPs, especially MMP-7, could be complementary indicators in the diagnosis of advanced fibrosis, does it mean it could be served as a diagnosis biomarker of advanced fibrosis?
Answer: We believe that the concentration and activity of MMPs can be used to indicate fibrosis progression. Also, we consider that MMPs could be as a therapeutic target, because those proteinases are directly involved in the degradation of the extracellular matrix. Our studies are currently focused on identifying the specific serum proteins that would improve the available diagnostic methods, or perhaps design a novel panel to discriminate the fibrosis degrees, according to liver insult. We added that idea to the manuscript to emphasize the reviewer's comment: "The use of MMP-7 as a complementary protein could improve the specificity and sensitivity of the available methods for determining this fibrosis stage".

Science editor

We truly appreciate all your observations, which undoubtedly will increase the quality of this paper.
Additionally, we added an Article Highlights section at the end of the main text as the *Science editor* mentioned

Article Highlights

Research background

Metalloproteinases (MMPs) maintain the homeostasis between fibrogenesis and fibrolytic processes in the liver. Few studies on the production and activity of liver MMPs and fibrosis progression have been performed in humans.

Research motivation

The correct determination of liver fibrosis stages is imperative for making the diagnosis and implementing therapeutic decisions. At present, there is no evidence of the production and activity of MMP-2, MMP-7, or MMP-9, or their correlation with fibrosis progression, in serum samples from patients with liver diseases.

Research objectives

In the present prospective, cross-sectional, multicenter study, we assessed the production, activity, and regulation of metalloproteinases in liver fibrosis stages in Chronic hepatitis C (CHC).

Research methods

We selected CHC patients from the *Hospital General de México, "Dr. Eduardo Liceaga",* the *Universidad Autónoma de Nuevo Leon*, and the *Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán".* Patients were categorized in fibrosis grades through FibroTest® and/or FibroScan® (F0, F1, F2, F3, or F4). Serum concentration of MMP-2, -7, and -9 were determined. Differences were validated by the Kruskal-Wallis and Mann-Whitney U tests. Area under the receiver operating characteristic (AUROC) curve was calculated in fibrosis degrees. Proteolytic activity was validated by chromogenic and enzymatic assays and serum concentration, and the regulation of tissue inhibitor of metalloproteinases-1 (TIMP-1) was tested in fibrosis progression.

Research results

We compared 119 CHC patients with 119 healthy subjects. MMP-2, -7, and -9 concentrations were higher in the patients with CHC than in the control subjects. No differences between the serum concentrations of MMP-2 and MMP-9 were found, but MMP-7 showed differential regulation, in accordance with fibrosis stages, as well as an acceptable ROC (0.705), in advanced fibrosis (F4). Collagenolytic MMP activity was maintained in F0 and F1, but decreased significantly in F2, F3, and F4. Gelatin activity was not observed in any stage of fibrosis. The concentration of TIMP-1 was lower in F2 and F4, compared with F0, F1, and healthy subjects. Inactive metalloproteinases were found in the serum of the CHC patients.

Research conclusions

Elevated concentrations of inactive MMPs were present in the serum of CHC patients, reflecting the impossibility to restrain liver fibrosis progression. MMPs could be used in the diagnosis of liver fibrosis and the treatment for its reversal in CHC.

Research perspectives

Given that MMP-2, -7, and -9 have not been simultaneously evaluated in serum from liver fibrosis patients, MMPs could be used to improve the currently available diagnostic methods and as therapeutic targets. They could also be used as a monitoring tool in treatment-experienced patients that continue to present with liver fibrosis and develop cirrhosis and/or HCC.

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

(1) ***Science editor:*** 1 Scientific quality: The manuscript describes a clinical and translational research of the production and activity of metalloproteinases during liver fibrosis progression of chronic hepatitis C patients. The topic is within the scope of the WJH. (1) Classification: Grade B and Grade C; (2) Summary of the Peer-Review Report: The authors conducted a series of experiments to evaluate the serum concentration and proteolytic capacity of MMP-2, -7, and -9 in CHC patients, according to fibrosis progression. The experimental design is reasonable and the work is worthy of further study. However, the number of samples should be expanded. The questions raised by the reviewers should be answered; and (3) Format: There are 2 tables and 7 figures. A total of 40 references are cited, including 2 references published in the last 3 years. There are no self-citations. 2 Language evaluation: Classification: Grade B and Grade B. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the Clinical Trial Registration Statement, and the Institutional Review Board Approval Form. Written informed consent was waived. The authors should provide the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement. No academic misconduct was found in the CrossCheck detection and Bing search. 4 Supplementary comments: This is an unsolicited manuscript. The study was supported by National Council for Science and Technology and PAPIIT-UNAM. The topic has not previously been published in the WJH. 5 Issues raised: (1) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s); (2) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor; and (3) The “Article Highlights” section is missing. Please add the “Article Highlights” section at the end of the main text. 6 Re-Review: Required. 7 Recommendation: Conditional acceptance.

(2) ***Editorial office director:*** I have checked the comments written by the science editor.

(3) ***Company editor-in-chief:*** I have reviewed the Peer-Review Report and the full text of the manuscript, all of which have met the basic publishing requirements, and the manuscript is conditionally accepted with major revisions. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report and the Criteria for Manuscript Revision by Authors. Before final acceptance, authors need to correct the issues raised by the editor to meet the publishing requirements.

References:

Helaly GF (2011) Differences in circulating MMP-9 levels with regard to viral load and AST:ALT ratio between chronic hepatitis B and C patients. British journal of biomedical science 68(1):38-42 doi:10.1080/09674845.2011.11732840

Irvine KM, et al. (2016) Multiplex Serum Protein Analysis Identifies Novel Biomarkers of Advanced Fibrosis in Patients with Chronic Liver Disease with the Potential to Improve Diagnostic Accuracy of Established Biomarkers. Plos One 11(11):e0167001 doi:DOI:10.1371/journal.pone.0167001

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Thomas H (2018) Biliary tract: MMP7 - a diagnostic biomarker for biliary atresia. Nature reviews Gastroenterology & hepatology 15(2):68 doi:10.1038/nrgastro.2017.175

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