Dear Editor-in-Chief and worthy reviewers

Thank you for your useful comments and feedback on our paper which helped us to improve its presentation and quality. We have carefully addressed all of your comments in the revised manuscript. We hope that you will be satisfied with the response provided by us.

Sincerely,
The authors.

Reviewer 1’s comments

The study focuses on diagnosing chronic HCV infection using both invasive and non-invasive methods. The non-invasive indices, including serum fibronectin, APRI, AAR, and FIB-4, were analyzed in relation to Shear Wave Elastography (SWE), considered a gold standard along with invasive liver biopsy for liver fibrosis determination. Employing an Artificial Intelligence method, the study aimed to predict the severity of liver fibrosis and understand the intricate relationship between non-invasive indices and fibrosis severity. In a hospital-based study involving 100 untreated HCV-positive patients, statistical and probabilistic analyses were conducted. Notably, fibronectin exhibited high diagnostic accuracy in predicting fibrosis stages (mild, moderate, and severe), with sensitivity, specificity, and AUROC values indicating strong correlations. The Bayesian Network underscored the relationship between fibronectin, AAR, APRI, and FIB-4 and patients with severe fibrosis, leading to the conclusion that fibronectin strongly correlates with liver fibrosis progression in HCV patients. Additionally, the study affirms that the severity of liver fibrosis escalates with increasing values of the investigated non-invasive indices. As a reviewer, I would like to draw attention to several considerations in the methods section of the manuscript.

Response to comments:

The authors are thankful to the reviewer for the providing us the chance to revise the manuscript and provide our response to the critical issues raised by the worthy reviewers. As suggested, we have carefully addressed all the comments received from the reviewer. The point wise reply to the comments as per the reviewer report are provided in this rebuttal. We hope the revised manuscript would be considered for the publication.

Comment 1: The use of an alcohol use questionnaire for excluding NAFLD patients warrants further clarification, particularly regarding its effectiveness in ruling out NAFLD, especially in individuals with a BMI less than 25 who can still present with fatty liver. It is crucial to address this limitation and explore alternative or additional criteria for a more comprehensive exclusion process.
Response 1: The authors want to clarify that we have used questionnaire to rule out serious alcohol use. However, we diagnosed NAFLD based on metabolic syndrome criteria and ruled out patients with Type 2 diabetes mellitus (+ve), body mass index (≥ 25 kg/m²), and metabolic abnormalities (such as waist circumference (≥ 94/80 in men/women), blood pressure (≥ 130/85 mmHg), plasma triglycerides (≥ 150 mg/dL), HDL cholesterol (> 40/50 mg/dL for men/women), HOMA-insulin resistance score (≥ 2.5)). As suggested in the next comments, we have updated the above criteria as cardiometabolic criteria for ruling out metabolic dysfunction-associated steatotic liver disease (MASLD).

Comment 2: Furthermore, I strongly recommend updating the terminology used in the study to align with the new nomenclature outlined in the Global Nomenclature Initiative. The provided link: https://www.aasld.org/new-masld-nomenclature#:~:text=Global%20Nomenclature%20Initiative%3A%20New%20Nomenclature%20Announced&text=should%20be%20retained.-Nonalcoholic%20fatty%20liver%20disease%20(NAFLD)%20will%20now%20be%20metabolic%20dysfunction,of%20five%20cardiometabolic%20risk%20factors.serves%20as%20a%20useful%20reference%20for%20this%20purpose.

Response 2: Thanks for suggesting the new study. We updated the manuscript to mention the latest terminology based on cardiometabolic criteria for ruling out metabolic dysfunction-associated steatotic liver disease (MASLD).

Comment 3: Additionally, a discussion on the choice of Fibroscan over ultrasound for liver stiffness assessment is warranted, particularly considering the prevalence of Fibroscan in the USA.

Response 3: The authors want to clarify that we have used Shear Wave Elastography (SWE) in the current study to assess liver fibrosis. Imaging techniques such as fibroscan, transient elastography and Shear Wave Elastography (SWE) are non-invasive methods to assess liver fibrosis. Although Fibroscan is prevalent in USA, it has several limitations including cost of the equipment and lack of standardized cutoffs for the diagnosis of fibrosis stages. SWE is a non-invasive imaging tool that measures liver stiffness that, in turn, has been validated in clinical and research studies [10, 11] as a surrogate marker of liver fibrosis. This technique can help to gather real-time images through a B-mode ultrasound probe [9]. Liver fibrosis determination by invasive biopsy and non-invasive SWE agree closely; thus, both methods are considered to be gold standards [12, 13, 14]. Existing studies [15, 16, 17] strongly suggest that SWE is accurate and has diagnostic effectiveness in predicting and staging biopsy-proven liver fibrosis patients across varied populations worldwide.

The above discussion has been added to the revised manuscript.

Comment 4: Addressing limitations, such as the small sample size and the absence of diverse demographic characteristics, is essential for a comprehensive evaluation of the study's findings.

Response 4: Keeping in view of the availability and feasibility of the participants, a non-random convenient sampling technique was adopted. The authors have mentioned the sample size calculation technique and the associated limitation related to data size in the revised manuscript.

Furthermore, we want to highlight that the study was conducted for the Malwa population from Punjab, India that represents HCV hotspots compared to other parts of India. Here, the most common means of HCV transmission are related to blood transfusion and the insecure use of
injections for therapeutic reasons. Due to this reason, we have covered limited demographic characteristics. We have mentioned these aspects in the subjects.

Comment 5: Moreover, acknowledging the routine use of Fib-4 in the USA and discussing its implications in the context of the study would provide valuable insights for readers.
Response 5: We thank the reviewer for pointing this to us. We have mentioned details about FIB-4 in Section 2.2.2 and have provided equation used to calculate its value. Further, we have acknowledged the use and implications of FIB-4 in relation to the present study in results section and discussion section.

Comment 6: Furthermore, I recommend separating the Results and Discussion sections to enhance clarity.
Response 6: We thank the reviewer to highlight this issue. We want to clarify that the results and discussion are added as separate sections in the revised manuscript (Section 3: Results and Section 4: discussion).

Comment 7: The tables and graphics presented in the manuscript appear confusing and are challenging to follow, requiring a reevaluation of their presentation.
Response 7: The tables and figures have been revised and uploaded as separate files for the revised submission, and they are clear.

Comment 8: Regarding the Background section, it appears incomplete and lacks relevant information specific to the study's question.
Response 8: We have rechecked the background section and improved it to make clear and more comprehensive.

Comment 9: Additionally, the statement that HCV is diagnosed by fibrosis is inaccurate and should be corrected.
Response 9: Thanks for pointing this error. We have corrected the error.

Comment 10: A more accurate representation of HCV diagnosis should be provided to enhance the precision and credibility of the manuscript.
Response 10: The representation of HCV diagnosis is provided with accurate representation in the revised manuscript.

Comment 11: Are there any other medical conditions that affect fibronectin levels? More information should be included in the discussion section and how this could affect the results of this study.
Response 11: As suggested, we have discussed the effect of fibronectin levels on other diseases and how they can impact the present study. We have added some discussions on this aspect in the discussion section.