Peer Review File

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Reviewer Comments

This is an interesting paper that addresses an important topic. The subject matter is certainly relevant because HCC cases due to NAFLD are increasing during the last years, and the factors that determine recurrence after surgical resection, especially in this etiology, need to be identified for a closer surveillance in high-risk patients. However, several issues deserve consideration and should be taking into account to improve the manuscript

My comments regarding this study are as follows:

Major comments:

- The introduction and justification should be better structured and should guide the reader to the main purpose of this study.

1) The sentence "no effective therapy has been found for the treatment of NASH" is wrong, exercise and Mediterranean diet have proven to be effective, maybe the authors are trying to say that there is no still pharmacologic treatment for this disease.

Response

- Firstly, I would like to thank you for reviewing my manuscript and for your expert ideas and suggestions. I appreciate your valuable comments. As you have suggested, I have made changes in the paper and revised it. The sentence no effective therapy has been found for NASH treatment has been changed. As you mentioned we were talking about pharmacological treatment for NASH.
- After revision: No effective pharmacological therapy has been found for the treatment of steatohepatits

2) The authors should focus their justification on known prognostic factor for recurrence after HCC resection and describing the main immunohistochemical features that have been demonstrated their implication in HCC prognosis, or specifically for recurrence after resection

Response

- Thank you for your suggestion. We have looked into data regarding other immunohistochemical markers and included a sentence regarding the same in the introduction
- After revision: Some of the factors previously identified as prognosticators for HCC are serum AFP level, des-gamma-carboxy prothrombin level, tumor size and number, margin status, major vessel invasion, tumor stage, Edmonson-Steiner grade, Child-Pugh score, portal hypertension and cirrhosis. Immunohistochemical markers studied are keratin 19 (K19), epithelial cell adhesion molecule (EpCAM), and CD133.

3) It should be considered that no previous studies have been shown the influence of the etiology of HCC in the risk for recurrence after resection (however, previous studies showed a little percentage of patients with cryptogenic or NAFLD etiology)

Response:

- Thank you for your comment. We have added this statement in the introduction as you suggested
- After revision: No previous studies have shown the influence of the etiology of HCC in the risk for recurrence after resection.

4) Methods

The number of patients should be indicated in the results section, not in the methods section

Response:

- Thank you for your suggestion. We have indicated the number of patients in the results section instead of methods section.

5) The fact that there was only a pathologic that evaluated the samples is a clear limitation of the study, how many pathologists were involved in the evaluation of the samples?

Response

- I agree that a pathologist review alone is a limitation of our study. We had two pathologists who reviewed our slides.
- After revision: All specimen slides were evaluated by two pathologists.

6) Why the authors selected a p value of <0.1 for significance in the NAFLD group?

Response

- Thank you for raising this point. In evaluation of NAFLD patients a p value of <0.1 was selected for significance as very few factors showed a significance level of <0.05 so could not be used for multivariate analysis hence a value of p<0.1 was selected.
- After revision: For the survival analysis of patients in the NAFLD group, a *P* value of <0.1 was considered significant as very few factors showed a significance level of <0.05 so could not be used for multivariate analysis,

7) It would be more accurate to say “extrahepatic progression” instead of “systemic progression”

Response

- Thank you for your suggestion. Extrahepatic progression seems more appropriate instead of systemic progression
- After revision: Systemic RFS was calculated as the time from the date of surgery to the date of the first instance of extrahepatic progression

8) There was a limited sample size in the NAFLD group compared with HBV etiology which hampers the interpretation of the obtained results

Response

- We agree that the number of patients in the NAFLD group being small is a limitation in our study. However, even though number of patients with NAFLD associated HCC are increasing the overall number is not very high world over and a timely evaluation of outcomes of NAFLD associated HCC was required hence we went ahead with our evaluation.
- After revision: The retrospective design of this study and the limited sample size, especially of patients with pure NAFLD, are the major limitations of this study. However, even though number of patients with NAFLD associated HCC are increasing the overall number is not very high world over and a timely evaluation of outcomes of NAFLD associated HCC was required hence we went ahead with our evaluation.

9) In addition, authors should analyze separately the cirrhotic and non-cirrhotic patients because the cirrhosis is a well-known prognostic factor for HCC recurrence after resection and it is an important bias of this study.

Response

- This is a very relevant point. Cirrhosis is an important prognostic factor for HCC, however we were not able to analyse the patients with and without cirrhosis because of further reduction in number of patients in the NAFLD group leading to censoring of patients and inadequate results.
- After revision: Cirrhosis is an important limiting factor in the study leading to bias. Since evaluation of patients with NAFLD and cirrhosis reduced the patients further we were not able to accurately analyze this subgroup.

10) The number of nodules is a variable not recorded.

Response

- The number of nodules has not been included as a continuous variable in the analysis , however it has been added in the form of a categorical variable as solitary versus multiple nodules in the evaluation.

11) For OS and RFS analysis, the period of follow-up should be specified, at 5-years? What was the median of follow-up.

Response:

- The median follow-up duration has been indicated in the factors affecting survival part of the results section.
- After revision: Patients had a median follow-up of 42 months (21-75).

12) The authors wrote “although this did not reach statistical significance”. However, if the results is not statistical significant the authors should omitted this clarification.

Response

- Thank you for your comment.
- After revision: As suggested, the statement “although this did not reach statistical significance” was deleted.

13) Another relevant issue is that Ki-67 is a predictor of recurrence and survival for all groups and in the NAFLD group, however, there is no data about predictors in the HBV group (it is ki-67 a specific predictor for NAFLD patients??)

Response

- Ki67 is a predictor for patients with HCC. Previous studies have shown the significance of Ki67 as a marker for overall survival and recurrence in HCC associated with different factors and their results were based on a mixed population. Hence we decided to study ki-67 as a predictor for patients with NAFLD associated HCC and did not analyse patients in the HBV group in this respect.
- After revision: Several studies have shown that the Ki-67 labeling index is a prognostic factor for recurrence and OS in patients with HCC. However, most of these earlier studies included mixed populations. The molecular markers for prognosis may differ between the different etiologies of HCC, including HBV, HCV, NAFLD,

and alcoholic liver disease. Hence, studies involving mixed populations may provide inaccurate results. Hence we decided to study ki-67 as a predictor for patients with NAFLD associated HCC and did not analyse patients in the HBV group in this respect

14) Minor comments

For figure 1, not only extrahepatic recurrence should be represented. Recurrence, local and extrahepatic and OS could be shown.

Response

- Thank you for your suggestion. I have added the figures representing recurrence, local and extrahepatic. An additional figure (figure 2) has been added representing this.

15) The total n of each group should be specified in tables, and specify n (%)

Response

- The total n of each group has been mentioned in the tables. I have specified the n (%) in table 1 as well.

16) All tables make reference to the predictors for survival. Nevertheless, RFS and OS are represented in tables, so titles should be better explained.

Response

- Thank you for bringing this to my notice. As you suggested, we have changed the title of table 1
- After revision: Pathological factors and recurrence data of patients in the HBV and NAFLD group.