ROUND 1

Lian-Sheng Ma

Company Editor-in-Chief

World Journal of Gastroenterology

Re: Manuscript #63387 (Review); Update on the association of Hepatitis B and intrahepatic cholangiocarcinoma: is there new evidence?

Dear Editor,

Thank you for your time to review our submission to your journal. Your suggestions and the reviewers' comments have been extremely helpful for the final development of our manuscript. Please find below (underlined) the changes that we have made to our initial submission in response to your suggestions and the reviewers' comments.

Reviewer #1 comments:

I think this manuscript is a polite review of many reports. I think the appearance of the manuscript is good. It has been investigated in detail, but could the following points be related?

1. Relationship with HBV genotype

2. Relationship with Occult HBV Infection If you have any reports, please add them.

Thank you for the points you have indicated, which are both very important. To address them, we added the following two paragraphs at the end of the "Epidemiological studies of HBV-associated iCCA" section:

"Occult HBV infection (OBI), which is defined by the detection of HBV DNA in the serum and liver tissue of HBsAg-negative individuals with or without serological markers of previous viral exposure[29], is an emerging risk factor for iCCA. Hepatitis B virus maintains its pro-oncogenic properties in patients with OBI[30]. Indeed, epidemiological and molecular studies have indicated that OBI represents an important risk factor for the development of cirrhosis and HCC[31,32]. Relevant evidence from pathological studies, investigating the presence of HBV DNA, genes and proteins in iCCA tissue specimens, also suggests a possible etiological role of OBI in iCCA[33–35]. A case-control study of 183 cryptogenic iCCA patients and 549 healthy individuals confirmed that HBsAg seroclearance does not signify eradication of HBV and may not entirely prevent the development of iCCA in Chinese patients[33]. Pollicino et al. investigated the presence of HBV DNA in liver tissue specimens from 47 HBsAg-negative patients with iCCA and 41 paired non-tumor liver tissues. A high prevalence of OBI in iCCA patients, along with the presence of both free viral genomes and integrated HBV DNA in the tumor tissue, suggested an involvement of HBV in the carcinogenesis of iCCA not only in overt but also in occult infection[34].

To date, there is limited data examining the relationship between iCCA and HBV genotype. Barusrux et al. investigated the prevalence of HBV and HCV infection among 295 CCA patients in northeast Thailand[36]. In this study, HBV genotypes C (73.3%) and B (26.7%) were detected, in line with the HBV genotype distribution in Thailand[37]. In a cohort of 11 cases of iCCA in the eastern United States, liver specimens were examined for HBV DNA and HCV RNA and the genotypes observed represented the

dominant genotype (genotype A) in this area[38]. Currently, there is no sufficient evidence to support an etiological association between specific HBV genotypes and iCCA."

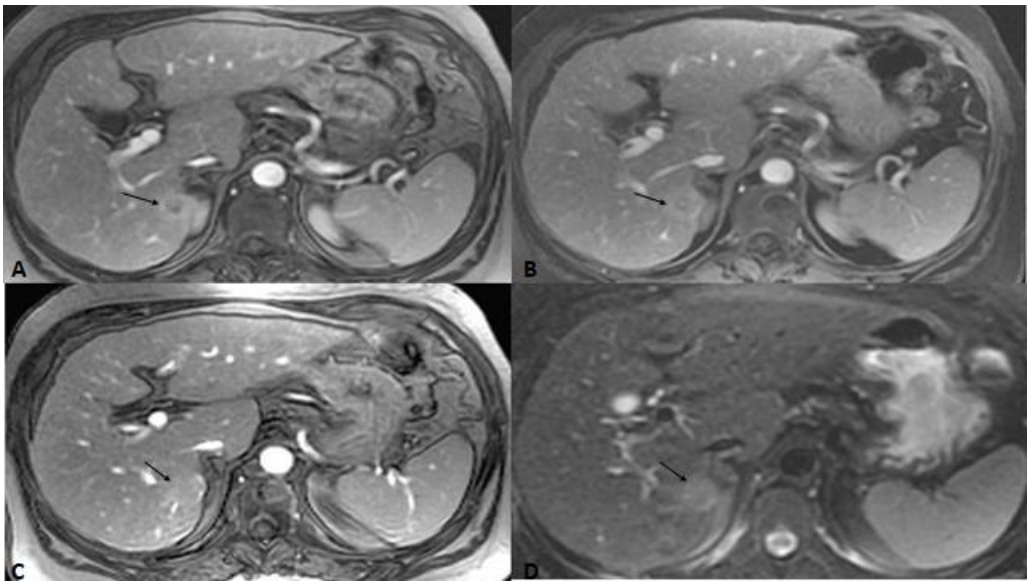
Reviewer #2 comments:

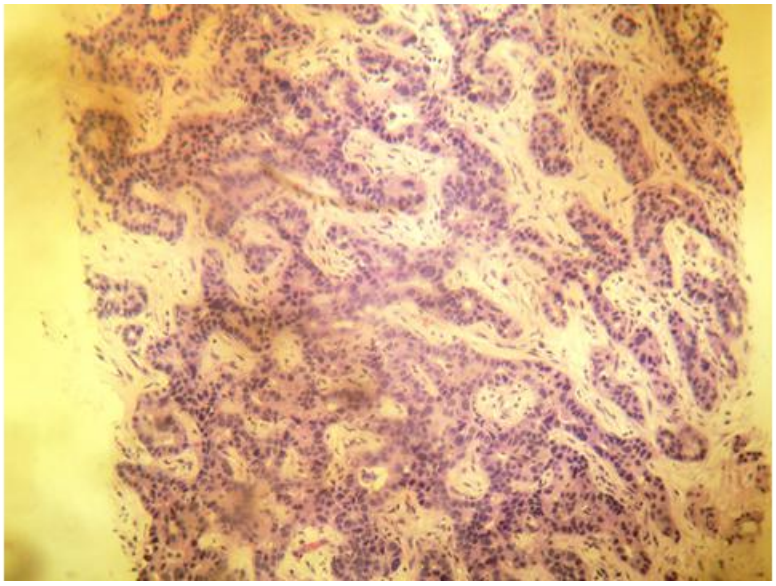
1. The areas with high prevalence of hepatobiliary cancer are all Asian countries, of which China is the high incidence area, but why is the Taiwan area of China a lower prevalence (in the epidemiological studies section)? Are there any relevant literature reports to support this view?

Thank you for your comment. This was actually a mistake. In the revised manuscript we have corrected the "classification" of countries including Taiwan in the countries with high or intermediate prevalence of intrahepatic cholangiocarcinoma.

2. Should the CT and histological findings of this clinical case be presented to better illustrate the close relationship between HBV and iCCA (The case in introduction section)?

We added relevant figures (Figure 1 and Figure 2) in the introduction as follows.





3. Should state the correlation between hepatitis B and iCCA in the future in conclusion section? Are there any prospects for mechanisms such as therapeutic targets, antiviral therapy, immunotherapy?

To address your point we have separated the "Conclusion section" in two paragraphs, the second one describing the future prospects for the management of HBV-associated iCCA as follows:

"In terms of management, the need for effective treatment modalities beyond SR is critical both in the first and the second line treatment. The

evolution of personalized approaches through the recognition of specific therapeutic targets will improve the effectiveness of our treatment approaches. It is also likely that the results of ongoing studies in the field of immunotherapy will allow the use of this promising treatment in patients with iCCA, as well. Furthermore, it is currently unclear whether antiviral treatment for HBV can decrease the incidence of iCCA. Large, well-designed studies should address this important question. Focused research on these aspects of the management of HBV-associated iCCA in the future will enhance our ability to manage successfully this dreadful cancer."

Reviewer #3 comments:

The manuscript by Fragkou N et al. summarizes the latest evidences on the possible association between hepatitis B virus (HBV) and intrahepatic cholangiocarcinoma (iCCA) development. The authors, through an exhaustive review of the literature, state that HBV infection increases the risk to develop iCCA in different population cohorts around the world, albeit not all the mentioned studies found thiscorrelation. Moreover, a pathological mechanism involving inflammation and viral gene expression could underlie carcinogenesis. Finally, a better overall survival is attributed to HBV-associated iCCA patients, since these tumors are usually diagnosed at early stages and therefore, amenable for surgical resection. Additionally, antiviral treatment has also exhibited some benefits for these patients. The manuscript contains some interesting data; however, there are some points to keep in consideration: Comments:

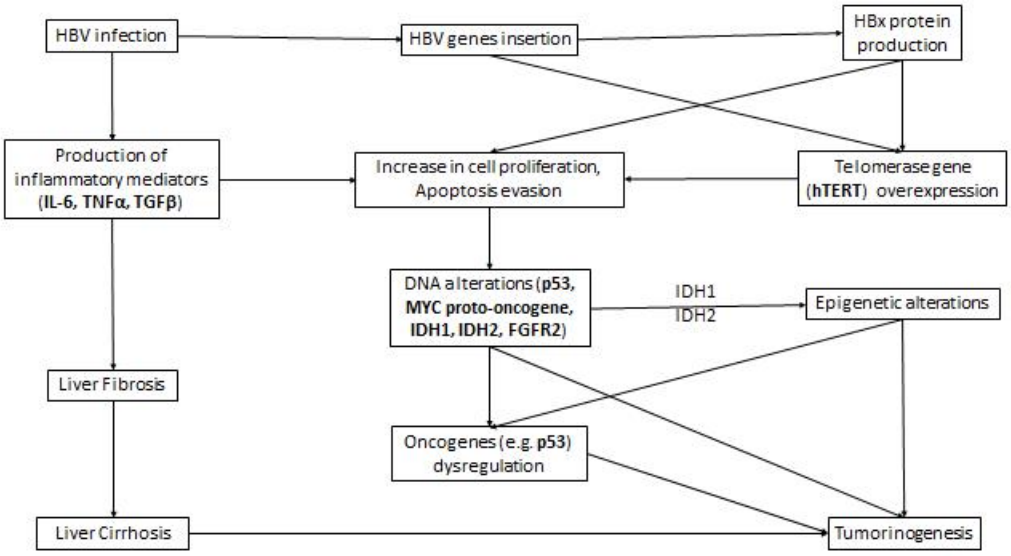
1. An "Abstract" section should be included.

We apologize for not including an abstract in our initial submission. This has been now added as follows.

"Intrahepatic cholangiocarcinoma (iCCA) is a subgroup of cholangiocarcinoma that accounts for about 10-20% of the total cases. Infection with Hepatitis B Virus (HBV) is one of the most important predisposing factors that lead to the formation of iCCA. It has been recently estimated based on abundant epidemiological data that the association between HBV infection and iCCA is strong with an odds ratio of about 4.5. The HBV-associated mechanisms that lead to iCCA are under intense investigation. The diagnosis of iCCA in the context of chronic liver disease is challenging and often requires histological confirmation to distinguish from hepatocellular carcinoma. It is currently unclear whether antiviral treatment for HBV can decrease the incidence of iCCA. In terms of management, surgical resection remains the mainstay of treatment. There is a need for effective treatment modalities beyond resection both in the first and the second line treatment. In this review we summarize the epidemiological evidence that link the two entities, discuss the pathogenesis of HBV-associated iCCA and present the available data for the diagnosis and management of this cancer."

2. A working model summarizing the potential pathological mechanism/s (with diagnostic markers) should be included.

In response to your comment, we have created a new figure (Figure 3) that summarizes the potential pathological mechanisms that lead to carcinogenesis in patients with hepatitis B and we have incorporated it in the section "Pathogenesis of HBV-associated iCCA".



3. The review explains the association between HBV infection and iCCA. However, there are some sections (e.g., management of unresectable disease and some sub-section of treatment of iCCA) where this association is practically absent, being only focused on iCCA. If possible it should be link this information with HBV infection.

We agree that the link between HBV infection and iCCA should be present throughout the manuscript including the treatment section. To make this evident, we added the following phrases in the section "Management of unresectable disease":

a) At the end of the sub-section "Systemic chemotherapy":

"A recent multicenter study used clinical and pathological data in addition to information from RNA sequencing in order to identify distinct subtypes of iCCA that might have favorable response to specific treatments. In the subtype that was associated with hepatitis B and C, an in vivo sensitivity to gemcitabine was observed. This was attributed to a higher expression of gemcitabine-response genes (SLC28A1 and SLC29A1)[137]. However, these findings should be confirmed in large

clinical studies prior to establishing a recommendation for patients with hepatitis B." and

b) At relevant points of the sub-section "Locoregional tretaments":

"A small, prospective study from China included 42 either HBsAg(+) or HBcAb(+) patients with iCCA who had received SR with curative intent and evaluated the prognostic impact of postoperative TACE[118]. TACE was performed in 9 patients who had demonstrated early recurrence of the tumor within 12 months from the surgery. The OS of the patients in the TACE (n=9) group was significantly prolonged (1-yr, 88.9%; 3-yr, 77.8%; 5-yr, 66.7%) as compared to the non-TACE (n=33) group (1-yr, 63.6%; 3-yr, 30.8%; 5-yr, 13%), implying that TACE could have a role in the management of patients with HBV associated iCCA when performed in the adjuvant setting."

"To our knowledge, there are no published studies regarding the use of ablative therapies in the context of HBV associated ICCA."

"Unfortunately, there are no published studies regarding the use of TARE in the treatment of HBV associated iCCA."

4. The manuscript should be thoroughly revised in order to correct typos.

Thank you for this comment. Author C.E., who is a native English speaker, corrected all typos found.

5. The abbreviations should be defined the first time they are mentioned in the text.

6. Numeric separators should be included.

Both comments # 5 and 6 have been addressed.

7. It should be interesting to explain some inconsistencies found between studies in order to a better understand.

Thank you for this important comment. We tried to explain the inconsistencies between studies by adding the following phrase at the end of the third paragraph of the "Epidemiological studies of HBV-associated iCCA" section:

"These seven studies were concluded prior to 2002 in geographically different areas. Geographical variations in iCCA incidence may be explained by differences in environmental and genetic risk factors. Moreover, observational studies are difficult to control for confounders, which could present an important risk factor and influence the prognostic value of HBV infection in iCCA."

We hope that the above mentioned changes satisfactorily address your comments and that our revised manuscript will now be approved for publication in your journal.

Sincerely,

Emmanouil Sinakos

ROUND 2

Dear Editor, Thank you for your time to review our submission to your journal. Your suggestions and the reviewers' comments have been helpful for the final development of our manuscript. Please find below (underlined) the changes that we have made to our initial submission in response to your suggestions and the reviewers' comments.

Editor and Reviewer comments: 1. I'm glad to see you revising your paper carefully according to these suggestions. "The population of the geographical regions of high or intermediate prevalence of cholangiocarcinoma including China and Taiwan" in the epidemiological studies section of the revised manuscript. However, Taiwan is a part of China, so the words should be changed to Mainland and Taiwan area of China.

Thank you for this clarification. We changed the words throughout the epidemiological section accordingly.