Format for ANSWERING REVIEWERS

November 22, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14695-Revised with Track Changes.docx).

Title: Primary biliary cirrhosis-associated hepatocellular carcinoma in Chinese patients: incidence and risk factors

Author: Xue-Xiu Zhang, Li-Feng Wang, Lei Jin, Yuan-Yuan Li, Shu-Li Hao, Yan-Chao Shi, Qing-Lei Zeng, Zhi-Wei Li, Zheng Zhang, George K.K. Lau, and Fu-Sheng Wang

Name of Journal: *World Journal of Gastroenterology*

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On behalf of all authors, I appreciate you and the reviewers very much for you instructive suggestions and the expert comments on our manuscript entitled as " Primary biliary cirrhosis-associated hepatocellular carcinoma in Chinese patients: incidence and risk factors". We also appreciate the comments from the reviewers which have helped us to improve our manuscript. As suggested, we sent our manuscript to an English-editing company (**American Journal Experts** **http://www.journalexperts.com/**) to improve the quality of the manuscript for the second time, and win the editing certificate granted by American Journal Experts (the current **Certificate Verification Key** is **F145-F6D7-E475-A17C-ADBB**). In addition, the revised parts have been highlighted with red color according to your suggestions and the comments raised by reviewers.

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated.

Thank for the suggestions of the editor. We have already modified the title of manuscript from 18 words to 12 words and the format according to the Journal's WRITING REQUIREMENTS OF CASE-CONTROL STUDY (ORIGINAL ARTICLE). Additionally, George K.K. Lau revised this paper and gave suggestions about answering reviewers, so he was present as a co-author. Ping-Ping He revised the statistical methods, so we acknowledged her in the paper. Li-Feng Wang was modified as a co-corresponding author for his contributions to this manuscript. The institution of the first author and the corresponding author has been modified as the requirements of the journal and Peking University.

2 Revision has been made according to the suggestions of the reviewer.

Reviewer No. 54593:

Comments: This is a well-written paper. There are a number of questions for the authors to respond. **(1)** Is there any policy in doing liver biopsy to stage the degree of liver cirrhosis and activity of inflammation in authors' centre apart from regular imaging? **(2)** More than 60% of tumors are greater than 3cm and multiple but according to the centre's policy, regular imaging was done every 3 to 6 months. How can the authors explain? An earlier stage of disease is expected. **(3)** How many patients are referred to liver transplantation during the follow-up period? What is the percentage of HCC (or incidental HCC) within this group of patients? It is important because it may have significant number and the incidence of HCC may be under-estimated. **(4)** Why 77 patients were matched in this case-control study?

Answer: Thanks for your very nice suggestions.

(1) Apart from regular imaging detection to stage the degree of liver cirrhosis and activity of inflammation, liver biopsy is another important choice. In our hospital, liver biopsy is mainly performed in patients whose etiology is unknown or the efficacy of treatment is not satisfactory if patients can tolerate liver biopsy and sign an informed consent. In our study, following the diagnostic criteria, PBC were established when two of three criteria were met [PMID: 19554543]. Respecting for the patient's own choice, the liver biopsy is not

necessary for PBC diagnosis. Therefore, clinicians regularly identify the degree of cirrhosis and activity of inflammation by serological indexes or combination with imaging. As you suggested, liver biopsy is more accurate to confirm the stage of liver cirrhosis and inflammation of liver. Right now, clinicians are making efforts to persuade more patients to accept liver biopsy in our hospital.

(2) It is really a good suggestion to enroll early stage patients to do the analysis. As reviewer mentioned that after patients were diagnosed as PBC, regular imaging was done every 3 to 6 months, the tumor should be found much earlier. However, in our hospital, the fact is that 61.54% of HCC associated PBC patients were diagnosed with PBC and HCC simultaneously or HCC diagnosis prior to PBC on their first medical consultation (multiple and greater than 3 cm tumors have already occurred at that stage) (Page 11, Line 6-8). This data is important in our paper; we wanted to show that PBC is not rare in china and should diagnose it as early as possible before relative tumor occurs.

(3) We checked all data of patients receiving liver transplantation in another unpublished paper from our group, and only two PBC-associated HCC patients underwent liver transplantation (LT). The liver transplantation rate was 3.85% in PBC-associated HCC group. The reviewer suggested a very important opining that those PBC patients who underwent liver transplantation may develop HCC if without LT, which makes the incidence of HCC under-estimated. We agreed with it and added it to our discussion (Page 13, Line 16-18).

(4) According to the principle of case-control study design, four controls were selected for each case matched by gender, age, follow-up period, and Child-Pugh scores. At last, 18 of 20 patients in case group could be matched by four controls, respectively. However, we could not find enough matched controls for two cases, so we used two controls for one case, and three controls for another ($18\times4+1\times2+1\times3=77$). It is the reason why 77 controls were included in our case-control study [PMID: 17174610].

Reviewer No. 1422704:

Comments: The authors present a case-control analysis based on very small numbers - only 20 cases. Therefore, it is not surprising that there were few positive findings. The authors need to acknowledge the severe limitations of this study. Also, p values should be

given exactly and not stated as p<0.05. There seems to be some contradiction where p values are quoted as more than 0.05 and an effect is regarded as being significant - this is not a correct way to describe these results. A thorough revision is required.

Answer: Thank you very much for your helpful and constructive suggestions.

We totally agree with you and acknowledge that 20 patients were not enough to get more positive findings and also described this limitation in our discussion part (Page 15, Line 15-17). In fact, the incidence of PBC is low (about 2.7 and 40 per 100,000, respectively) [PMID: 11113084], and the incidence of PBC-associated HCC is even lower [PMID: 9362353, 11981767, 17014578, and 23197466]. 17 cases and 24 cases of PBC-associated HCC were reported by Keith Lindor [PMID: 17174610] and Cavazza A [PMID: 19585656] respectively. So, to find PBC-associated HCC patients is not an easy work. In our study, we checked and followed up 1255 PBC patients from 2002 to 2013 in our hospital, 52 PBC-associated HCC patients were found. Among them, 20 patients met the inclusion criteria of the case-control study. Frankly to say, those cases were all we could find. By the way, our study still keeps on going, more PBC-associated HCC patients will be found, and more positive findings may be discovered in the future as you suggested.

According to your suggestion, we have already checked all the data involving *P* values in the manuscript and listed detailed P values accordingly. In addition, as you mentioned that there existed some contradiction between *P* values and significant effect for some results in our paper, such as the survival rate and age at HCC diagnosis between two genders (Page 11, Paragraph 2). Their differences have been reported to be significant by other groups. In our study, we could also see the difference between two genders but there was no statistic significance. In order not to miss the important information limited to the sample size, following your suggestion, we corrected the description and added "trend" to emphasize it, accordingly(Page 11, Line 9-13/15-17).

Reviewer No. 1560058:

Comments: In this case-control study, Zhang et al. examined the epidemiology of PBC-associated HCC in Chinese population. Furthermore, they investigated to determine risk factors for the development of PBC-associated HCC. The results suggested that a high BMI and a positive history of alcohol intake may be novel risk factors. The Reviewer has

the following comments. **Major points:** In Discussion, the authors speculated that a high BMI and a positive history of alcohol intake may synergistically enhance the progression of PBC and thereby increase the risk for the development of PBC-associated HCC. Did the patients with HCC actually had more advanced liver cirrhosis than those without HCC? The authors should compare between the two groups at the time of the diagnosis of HCC. **Minor points:** 1. Child-Pugh grade or scores and follow-up periods should be described in Table 3. 2. Please define significant digits in Tables. 3. Patients at risk should be added in Figure 3.

Answer: Thanks for your very nice suggestions.

Major points: As you mentioned, the severity of PBC did impact on the development of HCC. In clinic, the severity of PBC is often evaluated by the presence of liver cirrhosis and its complications, which are indicated by Child-Pugh scores. Following your suggestion, we compared the severity of PBC by Child-Pugh scores and liver cirrhosis at the time of the diagnosis (as shown in Table 3), and the results showed that there was no statistical difference between two groups.

Minor points:

1. Child-Pugh scores and follow-up period has been added in Table 3 according to your suggestion.

2. It is really a good suggestion, and we have already defined significant digits (red color) in Tables.

3. You gave us a valuable suggestion. We analyzed the impact of risk factors (history of alcohol intake and BMI $\geq$ 25) on the survival of PBC-associated HCC patients, but there was no significant difference (see Page 12, Line 11-13; Supplemental Fig 1).

Reviewer 31305:

Comments to authors: Interesting paper that shows risk of HCC in PBC patients. I have some minor suggestions, in the beginning of discussion authors should briefly give information about development of HCC and extra-hepatic malignancy in other autoimmune liver diseases including AIH, PSC and overlap of these conditions (Ngu JH, Hepatology. 2012 Feb;55(2):522-9.Ozaslan E,Clin Gastroenterol Hepatol. 2014 May;12(5):863-9.)

Answer: Thanks for your helpful and positive comments. We have given a brief description about development of HCC and extra-hepatic malignancy in other autoimmune liver diseases, which makes the discussion part more comprehensive.

3 References and typesetting were corrected.

We believe that our improved manuscript is of merit and the paper is worthy of publication in a high quality peer reviewed journal such as yours.

Thank you again for publishing our manuscript in the World Journal of Gastroenterology.

Sincerely yours,



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Reviewed by Journal Chief Editor Nahum Mendez-Sanchez:
It's a nice study. As authors mentioned the most important limitation of this study is the liver biopsy. I would like to sugggest to include in the introduction section la prevalence of PBC in China.

Answer: The suggested content have been added.