Author's response to reviews

Title: Prevalence of metabolic abnormalities in HBV related hepatocellular carcinoma in Chinese

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Author's response to reviews: see over
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Hello, Dear Editors:

Thank you for your kindly offering the chance for revising our manuscript and also the important suggestion for further improving our work!

In the revised manuscript, we had intensively adapted the discussion and the title according to the suggestions from the reviewers. The conclusions section, the competing interests section and the contributions section were added at the end of the manuscript, before the reference list. A statement of ethical approval had been added in the Methods section. Since all the samples we used in the research came from the department of laboratory medicine after routine detection. No additional in vivo research involved and the online registration is unnecessary. We had several publication with such kind of research [Int J Caner 2010;127:148-59, Human Genetics. 2010;127(1):75-81, Human Immunology. 2010;71(1):83-7, Int J Colorectal Dis 2010;25(1):39, Cancer Immunol Immunother Cancer 2009;58(9):1433-40]. All the revised part has been unlined as required.

In the next pages of this letter we have answered the reviewers’ comments point by point.

Thank you again for this revision chance and we are looking forward to hearing from you!

With best regards!

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Reviewer's report 1

Major points:
1. The main study design and results were not novel. Similar findings had been published.

Generally speaking we agree that the main study and results of this work were not novel enough, but as was pointed out by the reviewer himself, “the association of MDA (a marker of oxidative stress), GA (a maker of glucose metabolism) and free fatty acid with the development of HCC was also investigated in this study. This part of study finding was novel”.
In addition, the prevalence of MS and its impact on chronic hepatitis B (CHB) have just been focused recently, much remains to be done.

2. The enrolled population was quite heterogeneous and the enrollment criteria were not clear. Besides, the clinical, virologic and histologic features of the HCC patients were not presented clearly. The process of hepatocarcinogenesis is very complex. Both host and viral factors may be involved; it is important to evaluate known viral and host factors apart from the metabolic factors. For example, the baseline HBeAg status, the presence of fibrosis stage, the presence of liver cirrhosis, and serum HBV DNA should be provided.

We have modified the “Subjects and measurements” of the manuscript according to the suggestion of the reviewer. More detailed information regarding the enrolling criteria, the viral factors (HBV serum marker and HBV-DNA,) and the presence of cirrhosis had been added in the manuscript.

3. The cases and controls were not matched.

Yes. It is a cross-sectional study. Since the incidence of HCC is commonly identified in male and elder population. While inactive HBV carriers are often found in the younger. So the selection bias is difficult to control clinically. We have pointed out this disadvantage in discussion. In addition, in order to minimize this shortage, we have analyzed according to age and sex strata. As a result the unmatched case and control did not affect the conclusion we drawn (see supplementary data). On the other hand, the conclusion in this study have been adapted to be more conservative in description in revised manuscript due to the cross-sectional study character.

4. The sample size was relatively not large.

Yes. As was discussed in the manuscript, the study is the beginning of our whole project, we will validate our findings in larger sample size in future. So the conclusion in this study has been described in a more conservative way.
5. The conclusions can be softer, because the evidence in this study was not solid.

Yes. We have modified the conclusion.

6. The discussion part was tedious and too many review content.
The discussion had been cut shorter and re-organized more clearly.

7. Ethical issues were not addressed.
The ethical issues had been addressed in the revised manuscript.

Quality of written English: Needs some language corrections before being Published

The language had been polished by an English expert with scientific background.

Reviewer’s report 2
Major Compulsory Revisions
1. Statistical analysis. The Author used Tukey test in the analysis. However, this test requires observations to be independent, normally distributed, and to have homogeneity of variance (homoskedasticity). Usually, metabolic parameters do not have all these properties. Therefore, I think that a non-parametric test (e.g., Wilcoxon test) would be more appropriate. Alternatively, Tukey test could be used after a formal verification of its assumptions.

We have checked the statistical analysis carefully according to the comment. Firstly, we used One-Sample Kolmogorov-Smirnov to verify the data normality. Then Tukey test was used when the indicators following a normal distribution . Non-parametric data were analyzed with a post hoc analysis using Kruskal-Wallis H test. Categorical variables were compared using a Mann-Whitney U test. For analysis of the correlation between two indicators, spearman correlation analysis was performed. For multivariate analysis, multivariate logistic regression analysis was applied.

2. Results, 2nd paragraph. The sentence "The higher BMI, the higher..." seems not supported by the data reported in Table 2.

One parameter (ALT) which was not supported by table 2 we deleted from this sentence and also we change the description as to be understood more clearly. Sorry for the careless!

3. Discussion, last paragraph. Taking into account the case-control nature of the
study, reversal causation (i.e., the fact that metabolic abnormalities were consequences of HCC or HBV infection) should be discussed.

**We have modified the discussion intensively.**

4. Table 2. It is not clear whether the p-value reported in the tables refers to differences between strata (e.g., BMI < 24 vs BMI >= 24) or between study groups (HCC cases vs healthy controls).

The p-value reported refers to differences between strata (BMI<24 vs BMI≥24, GA ≤16% vs GA >16%, FFA≤0.6 vs FFA>0.6) in HCC group.

5. Tables 3 & 4. When evaluating a correlation between to variables through Spearman coefficient, the magnitude of the correlation should also be considered. Indeed, r<0.3 shows a small correlation between two variables, even if p-value is below 0.05. For instance, MDA cannot be considered strongly correlated to FFA or HDL (Table 4).

**We have modified the results and discussed in a more conservative way.**

6. Figure 1. It was surprising to see a significant difference in TC level between healthy controls and HCC cases. The same is for TG level between HBV-positive subjects and HCC cases. Indeed, the very high variance (as shown by error bars) should drive toward non-significant differences. To better understand the figure, the Author should: a) report the test used for comparison; b) specify what the bars represent (standard deviation or standard error?).

The comparison was performed by post hoc analysis using Tukey test in Figure 1. We had verified the data normality. The bars represented standard error. There were significant differences in TC and TG levels among healthy controls, HBV carriers and HCC cases. This result was intensively discussed in the revised manuscript. Similar researches supporting our findings had been cited in the discussion (see discussion paragraph 4)

7. Figure 2. In the figure, a linear model was applied to evaluate the relationship between tumor size and TG/GGT level. The linear model assumes a dependent relation between a dependent variable (TG or GGT level) and an independent variable (Tumor size). Therefore, in its present form, the model evaluates the influence of tumor size on TG/GGT level rather than the influence of TG/GGT level on tumor size. R-square is, however, very low, suggesting that other factors (other than tumor size) impact on TG and GGT levels.
Yes, Thank you for your comment!
The linear model assumes a dependent relation between a dependent variable (TG or GGT level) and an independent variable (Tumor size). So the model evaluates the influence of tumor size on TG/GGT level. Indeed this is another evidence that the lower level of TG correlated well with HBV related HCC, as was revealed by the step-wise logistic regression in this study.

Minor Essential Revisions
1. The Author should check the consistency of notation and abbreviations in the text, tables, and figure. Moreover, I think that "healthy controls" would be more appropriate than "normal controls".

Yes, we have checked the consistency of notation and abbreviations in the text, tables, and figure carefully. In addition, "healthy controls" had been used instead of "normal controls" in the whole manuscript.

2. Methods, 1st paragraph. The Author should report whether HBV-carriers and healthy controls had been matched to HCC cases, and, if so, the criteria for matching.

We had included the enrolled criteria for all the subjects in “Methods: subjects and measures”. On the other hand, since it was a cross-sectional study, to minimize the unmatched case and control, we had analyzed the important parameters according to age and sex strata. As a result the unmatched case and control did not affect the conclusion we drawn (see supplementary data). In addition, we have pointed out this disadvantage in discussion and the conclusion in this study had been more conservative in description.

3. Statistical methods. All the covariates included in the multivariate logistic model as adjustments should be listed.

There were too many indicators in this study, in order to make it easier to understand, only those variables with significant effect were listed in Table 5, which were the result of stepwise regression analysis.

4. Results, 3rd paragraph. According to results reported in Table 3, ALT seemed not correlated with metabolic indicators.
5. Results, 4th paragraph. The results reported in the text do not match those shown in Table 5.

The consistence between the results and table 3 & 5 was checked and revised. We are sorry for the careless mistakes. Thank you!
6. Discussion, 6th paragraph. Please specify that “metabolic abnormalities were positively related to the increased risk of HCC among HBV-positive Subjects”.

We have intensively revised the discussion and concluded that “metabolic abnormalities are closely associated with the occurrence and development of HBV-related HCC” instead.

7. Tables. The Author should state the test used for the calculation of p-value by adding footnotes to the tables.

The tests used for the calculation of p-values were added in form of footnote for each table according to the suggestion.

8. Table 5. Please check column headings, as they do not match to text (see point 5).

We have revised the table 5 according to the comment.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published
The language had been polished by an English expert with scientific background.

Reviewer’s report 3
Reviewer’s report:
Major Compulsory Revisions
Level of interest: An article of importance in its field
Quality of written English: Not suitable for publication unless extensively edited
The language had been polished by an English expert with scientific background.

1. Research question
The research questions stated by the author are not consistent and unclear through out the paper. For example: in the abstract it said: This case-control study was conducted to investigate the prevalence of metabolic abnormalities in HCC and to probe the association between metabolic parameters and liver function so as to evaluate the role of metabolism in the pathogenesis of HBV-related HCC. However, in the introduction of the main text, it was: we designed a cross-sectional study to clarify the influence of metabolic abnormalities on the pathogenesis of HBV-related HCC.
So it is unclear which study design was adopted and whether the author aimed to
investigate the association between metabolic disorder and liver function or metabolic factors with HBV related HCC. The study should be designed to address the research question. Also, the authors should justify why they chose those two control groups, inactive HBV carriers and healthy control (assuming that this group was HBV negative). Probably, it would be clearer if the authors focus on one research question on investigation of the association between metabolic abnormalities and HBV-related HCC.

This study was a cross-sectional study for evaluation the association between metabolic abnormalities and HBV related HCC. We have adapted the manuscript title as: “Association between metabolic abnormalities and HBV related hepatocellular carcinoma in Chinese: A cross-sectional study”. Thank you for your important comment!

The occurrence and development of HCC are extremely complex. HBV infection is the main etiological factor of HCC in China and Asia. So if compared with healthy control only, the conclusion will lose the etiology base. Therefore we chose two control groups: HBV carrier (as diseased controls) and healthy controls. In addition, the enrolling criteria for all the subjects and the results, conclusions were described in a more pertinent way.

2. Methodology
There is a problem with study method as indicated above. The research question was not clear and the study was not well designed. In the abstract, the authors stated it was case-control study (page 2, last sentence in background section); however in the main text the authors indicated that it was a cross-sectional study (page 5, last sentence under background section). As the study design was not clear, it would affect sample selection.

It is unclear how those three study group were chosen? Did the authors make any effort to minimize selection bias? If it was case-control study, how subjects were matched?

As explained in the comment 1, this was a case-control study and we had adapted this from title, abstract to the text of manuscript.

For sample selection, the questions involved are important and the three reviewers had the similar concern. We have adopted the suggestion and more detailed information regarding the enroll criteria, the viral factors (HBV serum marker and HBV-DNA,;) and the presence of cirrhosis had been added in the revised manuscript. But since the incidence of HCC is commonly identified in male and elder population. While inactive HBV carriers are often found in the younger. So the selection bias is difficult to control clinically. We have pointed out this disadvantage in discussion. In order to minimize this shortage, we have analyzed according to age and sex strata. As a result the unmatched case and control did not affect the conclusion we drawn (see supplementary data). In addition, the conclusion in this study have been modified to be more conservative in description.
The selection of control was of concerned. The control group was recruited from routine physical examination in Eastern Hepatobiliary Surgery hospital. Patients came to this hospital for examinations were more likely to have Hepatobiliary condition than general population.

The healthy controls were recruited not from patients, but from routine staff physical examination of the University and the hospital.

3. Data collection and data analysis
Data were collected from medical charts; therefore missing data and differential errors would be an issue. It is also unclear at what stage data were collected for analysis. For instance, in HBV-related HCC group, data on test results (e.g. liver functions, metabolic parameters) were collected at admission, or during hospitalization?

The information of weight, height, blood pressures, tumor size and whether there were any violations of metastasis, medical history, life style characteristics and other related information were collected from medical documentation. All the data in HCC patients on test were collected during hospitalization. All the blood samples were collected before surgery. Blood specimens were drawn after 12h of fasting and were subsequently measured at the Department of Laboratory Medicine in EHBH. With similar way, we have finished several works which had been published in: Int J Caner 2010;127:148-59, Human Genetics. 2010;127(1):75-81, Human Immunology. 2010;71(1):83-7, Int J Colorectal Dis 2010;25(1):39, Cancer Immunol Immunother Cancer 2009;58(9):1433-40.

The author concluded that Serum triglycerides (TG) and low-density lipoprotein cholesterol (LDL-cholesterol) levels were significantly associated with reduced risk of cancer (OR=0.05, 95% confidence interval, 0.01~0.27 and OR= 0.32, 95% confidence interval, 0.11~0.95, respectively). This conclusion needs to be seriously reviewed before publishing. As mentioned above, selection bias is major problem in this study. Data analysis needs to be revised. Some of the variable was claimed as significant different but the 95% CI was overlaps between groups (e.g.Figure 1). Also, which variables that were controlled for in the table 5 were not indicated.

The conclusion that serum triglycerides (TG) and low-density lipoprotein cholesterol (LDL-cholesterol) levels were significantly associated with reduced risk of cancer has been intensively discussed in the revised manuscript. Similar research supporting our findings had been cited in the discussion (see discussion paragraph 4). Table 5 has been revised.
4. Presentation, language
The manuscript was not well written. It needs to be edited by a native speaker before resubmitting. Structure should follow a structure of a scientific paper. There should be no text under figure legend except footnote if needed (e.g. there is more than a half of page of text under Figure 1). The title does not reflect the objective of the study as well as the content of the paper.

The language had been polished by an English expert with scientific background. The title had been adapted too, according to the comments.