Author's response to reviews

Title: Association between hepatitis B virus infection and metabolic syndrome: a retrospective cohort study in Shanghai, China.

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

Please find enclosed our responses to feedback from peer reviewers. We appreciate the input and feel we have addressed all of the relevant concerns.

Sincerely,

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Reviewer 1 Comments (in BOLD)

1. Methods:

1.1 Did authors examine anti HBc antibodies in HBsAg negative patients? Positivity of anti HBc antibodies could influence of MS incidence (it was demonstrated in previously published studies)

We noticed that in some publications such as “International journal of Obesity (2008) 32, 474-480[1]” mentioned “those with natural infection of hepatitis B infection (anti-HBc(+)) had 58% higher risk for metabolic syndrome”, this was a cross-section study in university freshmen so it cannot decide the Temporal Relationship between anti-HBc and MS though there was positive association. Moreover, the serum level of anti-HBc antibodies is a dynamic parameter in progress of chronic HBV. HBcAg is an accessory protein from the precore region of the HBV genome, which is not necessary for viral infection or replication. In the beginning of cohort surveillance founded, only HBsAg and anti-HBs was detected, we did the anti-HBc antibodies detection for all subjects of this study in the end of the study period (Dec 31th, 2011). For consistence of study, we selected HBsAg as represent of HBV infection.

1.2 Figure 1, selection of subjects during the study: please add in this figure:
- How many patient HBsAg positive (without MS) and HBsAg negative (without MS) had been recorded in year 1991?
- How many patients were excluded from the study during follow up from both groups due to:
  - Hepatitis C
  - Liver complications (compensated and decompensated cirrhosis, HCC, liver disease associated death, others)
  - Cardiovascular and metabolic complicanations (myocardial infarction, stroke, sudden cardiovascular death, others)
  - Other complications including death from other reasons

We updated this information in the new figure by reviewer’s request. In the HBsAg positive (without MS) group, there was 702 subjects were included in the beginning of study, 14 subjects were excluded due to hepatitis C and 43 subjects were excluded due to death then cannot gain any medical record, 27 subjects who matched the inclusion criteria refused to attend the study. In the HBsAg negative (without MS) group, there was 732 subjects were included in the beginning of study, 18 subjects were excluded due to hepatitis C and 41...
subjects were excluded due to MS, 129 subjects were excluded due to death then cannot gain any medical record, 49 subjects who matched the inclusion criteria refused to attend the study. We didn’t exclude any subject with liver complications, cardiovascular and metabolic complications if they were not match the definition of MS in the beginning of follow up. We believe in the cohort study if we exclude these subjects would result in selection bias then decrease the accuracy of study.

1.3 Had been patients really tested for HCV in 1991?

In 1991, there was not HCV test in China, so HCV positive subjects was recorded as non-A and Non-B hepatitis infection. From 1993, these subjects were tested then be confirmed as HCV infection.

1.4 Definition of metabolic syndrome

Why authors did use National Cholesterol Education Program Adult Treatment Panel # criteria from year 2001? Please to explain in text of this chapter.


“As a retrospective cohort study, all necessary data was tracked and collected in 2012 via database/medical record. In this type study, we need a good standard to determine disease status at present time though the standard didn’t exist in past, so we selected ATP III as the definition for metabolic syndrome to evaluate the association between metabolic syndrome and hepatitis B infection.”

2. Analyses of possible risk factors

2.1 Please to define

- Smoking,
- Passive smoking
- Drinking (Alcohol consumption)
- High-energy food intake
- Fresh fruits and vegetables intake
- Physical activity

These definitions had been included in manuscript.

“Definitions of smoking status for subjects were current smokers (if they were actively smoking) and previous smokers (if they had quit smoking for the past 6 months).

Definitions of passive smoking status for subjects reported their history of passive smoking exposure longer than 5 years.

Definitions of alcohol consumption status for subjects were current alcohol consumption (if they were actively drinking regardless of amount) and previous smokers (if they had quit drinking for the past 6 months).

Definitions of high-energy food intake were subjects who reported that they took fried, smoked, pickled foods and sweets over 5 times in one week.

Definitions of fresh fruits and vegetables intake were subjects who reported that they took fruits and fresh vegetables over 5 times in one week.

Physical activity was divided into 2 levels, none physical activity was defined as those who exercised less than 1 hour per week.”

2.2 Analyses of HBV infection associated with MS

Analysis is performed well, but I recommend to extend further statistics.

It would be very interesting to analyze, how does HBV infection influence not only MS, but also individual components of MS. I also recommend calculating crude age-adjusted hazard ratio between HBV infection and
- Increased waist circumference or BMI
- Hypertriacylglycerolemia
- Low HDL-C
- Blood pressure>130/85mmHg or current use of antihypertensive medications
- Hyperglycemia or antidiabetic therape use.

Analyses could be made independently for men and for women.

We considered conducting the analysis which recommended by reviewer when we drafted the manuscript, after various discussions within researchers we decided to move it from the manuscript based on two reasons listed below:

- Increased waist circumference or BMI, Hypertriacylglycerolemia and HDL-C is dynamic index for life style and it could vary in short period after behavior/life style changed, so if we calculated the association between HBV infection and these dynamic index may lead to unreliable estimation.
- Our retrospective cohort study followed up each subjects in different time point, how to define the positive components of MS in various timeline was a big challenge. For example, increased waist circumference may change in different followed up timeline, which one was the exact represent for subject?

So we used MS definition, a comprehensive index, as dependant variable to evaluate the association and ignored the association between its individual components and HBV infection. We believe this analysis may gain more reliable estimation.

To response reviewer’s question and try to provide more information for readers, we still calculated the estimation between individual components of MS and HBV infection, in the process we selected each component when subject was judged as MS if the subject was MS, the final followed up data was selected in the analysis if subject was not MS.

### Table 1 Components of MS and hazard ratios for group with or without HBV infection

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Male Crude HR† (95% CI)</th>
<th>Male Adjusted HR∆ (95% CI)</th>
<th>Female Crude HR† (95% CI)</th>
<th>Female Adjusted HR∆ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity (Increased waist circumference)</td>
<td>0.71 (0.12-4.26)</td>
<td>0.79 (0.13-4.94)</td>
<td>1.52 (0.76-3.03)</td>
<td>1.62 (0.81-3.24)</td>
</tr>
<tr>
<td>Hypertriacylglycerolemia</td>
<td>1.92 (1.17-3.16)</td>
<td>1.87 (1.13-3.08)</td>
<td>2.02 (1.09-3.71)</td>
<td>2.11 (1.14-3.90)</td>
</tr>
<tr>
<td>Low HDL-C</td>
<td>0.94 (0.38-2.32)</td>
<td>0.99 (0.40-2.47)</td>
<td>1.32 (0.68-2.57)</td>
<td>1.35 (0.69-2.64)</td>
</tr>
<tr>
<td>Blood pressure&gt;130/85mmHg or current use of antihypertensive medications</td>
<td>2.16 (1.33-3.53)</td>
<td>2.18 (1.33-3.56)</td>
<td>2.55 (1.44-4.50)</td>
<td>2.62 (1.48-4.64)</td>
</tr>
<tr>
<td>Hyperglycemia or antidiabetic therape use</td>
<td>2.09 (1.29-3.38)</td>
<td>2.07 (1.27-3.36)</td>
<td>1.56 (0.84-3.00)</td>
<td>1.59 (0.84-3.02)</td>
</tr>
</tbody>
</table>

† Adjusted for age; ∆ Adjusted for age; gender; smoking; passive smoking; drinking; high-energy food intake; Fresh fruits and vegetables intake; physical activity;
3. **Discussion need to be improved. I ask the authors to clarify, why they do find higher incidence of MS among HBV patients than in other studies (longer follow up? lifestyle differences in Puoto people? other?).**

In the discussion, we provided more details to clarify why our results was different with other previous study, we found longer follow up in our longitudinal study maybe the key factor for the difference. We improved the discussion based on reviewer’s suggestion and new reference recommended from reviewer listed below.

“Two cross-sectional studies in Hangzhou and Taiwan reported decreased risk for MS in subjects with HBV infection [20, 21]. A cross-section study in Hangzhou showed MS prevalence in subjects with chronic HBV to be 5.9% and 8.8% in the control group (OR=0.65, 95%CI: 0.48, 0.88) [21]. The same result was observed in the Taiwan Keelung Community-based Integrated Screening study, MS prevalence in subjects with chronic HBV and control subjects was 8% and 10.9% respectively (OR=0.84, 95%CI: 0.76, 0.93) [20]. In these studies, the criteria for defining MS were different from those of this study, and the prevalence of MS in HBV infected subjects may have been underestimated. Moreover, some potential confounders such as BMI and smoking were not adequately adjusted for. These studies were also limited by sample size and representation. The largest and latest study which included 593,594 subjects with chronic hepatitis B from NHANES III in US also reported an inverse relationship between chronic hepatitis B infection and MS[26]. This study was cross-section study design it included subjects from 2 months to elder. Two independent cross-section studies from Taiwan [27] and Slovakia [28] showed that there was no association between HBV and MS, in Taiwan’s study no individual components of MS was associated with HBV infection but opposite result found in Slovakian’s study that HBV infection may decrease level of TC and LDL. In other cross-section study from Taiwan showed that compared with healthy person, patients with chronic HBV infection had lower level of TG and LDL [29]. However these cross-section studies cannot examine the temporal association. In contrast, we used a retrospective cohort study based on a community population, and subjects with latent HBV infection received no antiviral therapy. Over an almost 20-year follow-up period, adjusted for some possible confounders, we evaluated the association between HBV infection and MS.”

4. **New articles studying association between HBV infection and MS should be cited in manuscript:**


These new references had included in revised version of manuscript.

**Reviewer 2 Comments (in BOLD)**

1. **The authors did not show results of the relationships between HBV infection and individual component of MS, especially lipid data. Several literatures reported the inverse association between HBV infection and lipid concentration, what about this study?**

We provided the calculation to response same comments for first reviewer and the reason why we didn’t include it in our manuscript (see answers for comments 2.2).

2. **What about body mass index? What’s the role of BMI in the association between HBV infection and MS? Although central obesity (indicated by waist circumferences) is one of the components**
in the definition of MS, studies showed that it is BMI, not central obesity, that is closely related to HBV infection. The authors need to clarify this.

We followed up the weight and height data for each subject. We noted that BMI is important determinants for the prevalence of fatty liver in Yuan-Lung Cheng’s study for health check-up subjects in Taipei, Taiwan [2]. In other study from Ming-Whei Yu in Taiwan, they found that excess body weight is involved in the transition from healthy carrier state to liver-related death among men [3]. In our study we just need a standard definition for MS to evaluate its association with HBV infection, we didn’t explore the relationship between obesity and HBV infection separately. We believe it would be good to use central obesity (indicated by waist circumferences) in our study though there were evidence showed BMI maybe associated with HBV infection. Moreover, we calculated the kappa index between BMI and central obesity, it was 0.92 (0.86-0.99).

3. The distributions of gender, age, education, race, family income are equally distributed between the exposed and nonexposed groups (as shown in Table 1). Is it coincidence? Or some matching process had been performed? What’s the coverage rate of the so called “population-based infectious disease surveillance system in 1991” in Shanghai? How did the authors determine the MS status at baseline? It does not look like they had performed any other tests other than HBsAg at baseline.

We would believe it was not coincidence. All subjects in nonexposed group were from similar community as subjects in exposed group, and they worked for similar government owner factory. As a retrospective cohort study, we didn’t conduct any matching process in our study. From 1980s, Shanghai founded some big government owner factories in Putuo area, and many people with similar backgrounds worked for these factories and lived in apartment build by government. Meantime, health care and serious infectious disease surveillance system was founded to serve these people. Almost all employees from these factories were cover by surveillance system. In surveillance system, it only had hepatitis lab test result. Fortunately all these big factories provided regular annually health check-up for all their employees so we could link the HBV infection data with EMR data via subjects’ unique ID. So in the beginning of study, we could determine the MS status for all subjects.

4. The authors stated in the “Methods” section that they started their Cox proportional hazards regression model with the adjustment of age and gender as “crude hazard ratios”, but in their Table 3, their “unadjusted hazard ratios” only adjusted for age (as indicated in the footnote).

We double checked our analysis protocol and original SAS program, in the Cox proportional hazards regression model only adjusted age as “crude hazard ratios”, we had revised the mistake in “Methods”. Thanks.

5. How about HBV viral loads and genotypes? Are there any dose gradients of HBV viral loads and the development of MS? Is there any genotype specific effect?

One study reported that “Viral load of chronic hepatitis B patients with MS was higher than that in patients without MS” [4], we thought HBV viral loads and genotypes may influence the association between MS and HBV infection. In 1991, only HBsAg been detected in the surveillance system and there was no available technology to measure HBV viral loads. As a retrospective cohort study, we only could track all available data to conduct the study so we don’t explore the influence of HBV viral loads, dose gradients and genotype specific effect in our study. This is the limitation of our study and we had discussed it in text. Thanks.

Minor comments:
1. Professional English editing is needed to correct numerous grammatical errors.

We found a professional medical writer to help us on English editing. Thanks.
2. The statistical methods used for the results presented in Table 1 and Table 2 were not stated in the text.

This part had been supplied in the text. Thanks.

“The data were presented as percentages for categorical variables as well as mean with standard deviations for continuous variables unless mentioned otherwise. Appropriate comparison tests including Chi-square test and Student’s t test were used for comparison between groups for categorical variables and continuous variables, respectively.”

Reference


