Author's response to reviews

Title: Multiplicative synergistic risk of hepatocellular carcinoma development among hepatitis B and C co-infected subjects in HBV endemic area: a community-based cohort study

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Author's response to reviews: see over
As of 15 Jul 2012

Dear Dr Upender Manne,

This refers the email dated on 16 June to revise manuscript. Thanks a lot all valuable comments and we believe that these very pertinent comments have helped us to improve our manuscript. The changes we made in response to reviewers’ comments are as follow. The changes were highlighted in the revised manuscript.

Response to the comments of Dr. M. Idrees

1. Better to change the title of the article as this does not reflect the results exactly.
   : As per reviewer’s comment, we have changed the title like below

   from “Hepatitis B and C coinfection and HCV genotype variation on hepatocellular carcinoma development: a population-based cohort study”

   to "Multiplicative synergistic risk of hepatocellular carcinoma development among hepatitis B and C co-infected subjects in HBV endemic area: a community-based cohort study"

2. Background needs to be updated by incorporating the latest references (2011-12)
   : As per reviewer’s comment, we cited the latest references in the background (reference 9-11, page 3).

3. Methodology is too long
   : As per reviewer's comments, we reduced Method section. See below item No 4 with details.

4. What was the method used to select the study population?
   ● The cohort study has conducted from 1993 to 2010 as a whole but in the present study we used and analyzed for cohort members enrolled from 1993 to 2003 only. To eliminate confusion, we deleted unnecessary explanation and cleared the study participants (page 4, line 21-22).
   ● All residents who live in Haman county and aged 30 years or older were invited the survey. The participants in the survey are recruited as cohort with informed consent. Haman county has relatively small population (70 thousand) and 66% of the population are 30 years or older. Around 10% of the whole population in the county voluntarily participated in the cohort study.
   ● After recruitment, we excluded subjects who did not meet the criteria for the analysis in the present study (e.g., cancer patients) as described in the Study participants in Methods section (page 4-5).

5. Complete demographic characteristics of the enrolled should be given.
   : As per reviewer’s comment, we changed table 1 to provide more complete demographic characteristics (page 20, Table 1).

6. No inclusion or exclusion criteria for the study subjects have been given.
   : Same as above (item number 4), the inclusion criterion for enrollment of the cohort was residents of Haman county. The exclusion criterion at enrollment was age under 30 years. The exclusion criteria for the analysis of the present study was those who reported existing cancer at enrollment, or who diagnosed cancer within 6 months of enrollment, or with incident non-Hodgkin lymphoma and cholangiocarcinoma (because these cancers are affected by HBV or HCV).

7. What is the prevalence rate of HCV & HBV in Korea?
   : The prevalence rate of HCV and HBV in Korea are 1.3-1.7% and 3.2%, respectively. (It was described in the
8. What was the study design? That is not cleared.

: The study design is the community-based prospective cohort design to estimate Hazard Ratio.

Please refer to the changed the title of the manuscript.

9. It is not mentioned in the results that what was sensitivity and specificity of the test methods used?

Please find information on sensitivity and specificity which are available in the reagent kit product (provided by commercial company).

: The sensitivity and specificity of EIA for HBV and HCV (AxSYM HBV version 2.0 & HCV version 3.0, Abbott Laboratory) used in the present study are very high.

- AxSYM HBsAg V2: sensitivity 100%, specificity 99.5% (package insert, 2004)
- AxSYM HCV version 3.0: sensitivity 100%, specificity 99.8% (package insert, 2004)

The sensitivity and specificity of NAT to confirm HCV RNA viremia (COBAS AMPLICOR HCV MONITOR test, version 2.0, Roche Molecular Systems) used in the present study are also very high.


We performed HCV genotyping with using Okamoto’s method for samples collected in 1990’s because commercialized LiPA had not been available in 1990’s in Korea. For samples collected in 2000’s, genotyping were performed with using LiPA which showed improved sensitivity.

We did not describe the sensitivity and specificity of each test because the Method section is too long.
Response to the comments of Dr Sheng-Nan Lu

General Comments:

1. Since this manuscript was submitted to this international Journal, you should not narrow your discussion in your country only. REVEAL study from Taiwan, a community-based study, published some papers on same issues. Please cite their publications and comparing your with them.
   : As per reviewer’s comment, recent papers of REVEAL study were added. (in the Background section, page 3, line 10; in the Discussion section, page 8, line 22- page 9, line2; page 10, line 13-15)

2. For the almost all Western and some Eastern studies reports the positive roles of DM and obesity in HCC development. In your study, the two factors were negative. You should emphasize the findings, enhance discussion of this part and add it in conclusion section.
   : As per reviewer’s comment, we added some interpretation in the discussion. (in the Discussion section, page 10, line 24-page 11, line 6)

3. Discussion was too long. It included well-known and unrelated issues. Some original findings and methods were not mentioned. Please see below comments
   : As per reviewer’s comment, we tried to shorten the discussion section, but some original findings and methods were inevitably added in discussion section.

Minor comments:

1. [Title] Population-based study means that whole, not a sample or partial, of population was included. This study was not a population-based study. It may be a community-based study.
   : As per reviewer’s comment, we have changed the title like below

   from “Hepatitis B and C coinfection and HCV genotype variation on hepatocellular carcinoma development: a population-based cohort study”

   to ” Multiplicative synergistic risk of hepatocellular carcinoma development among hepatitis B and C co-infected subjects in HBV endemic area: a community-based cohort study”

2. [Methods] There were two groups of study subjects were enrolled. One were during 1993~2004, and the other was during 2001 to 2010. There were a 17-year interval between 1st and the last of cases. During these 17 years, there were a lot of improvements of hepatology, including screening and diagnostic techniques of hepatocellular carcinoma (HCC) and treatment of hepatitis of B and C. Please describe these impacts in the study subjects, such as day of HB vaccine launching, status of reimburse of anti-viral treatment.
   : The cohort study has conducted from 1993 to 2010 as a whole but in the present study we used and analyzed for cohort members enrolled only from 1993 to 2003 to focus on a rural area, Haman county, showing high HCC incidence and mortality. To eliminate confusion, we deleted unnecessary explanation and cleared the study participants. (page 4, line 21-22)

   HBV vaccine has been nationally immunized for infants since 1995 in Korea. However HBV vaccination for adults and elders was not popular (vaccination rate in the study population was less than 30% and many of them did not complete three doses). Korea has universal health insurance system and all Korean citizens are covered by the national health insurance for liver diseases including anti-viral treatment. In 2003, National Cancer Screening Program for liver cancer launched and high risk group (i.e., HBsAg seropositive, anti-HCV positive or liver cirrhosis) can get sonography and AFP test for every 6 month for free. We did not describe this detailed explanation because the method section is too long.
3. [Methods] What is the different between determinant HCV genotype by Okamoto methods and Inno-Lipa? Did the viral load take into consideration? If not, it should be discussed as a limitation of study.

- We performed HCV genotyping with using Okamoto’s method for samples collected in 1990’s because commercialized LiPA had not been available in 1990’s in Korea. For samples collected in 2000’s, genotyping were performed with using LiPA which showed improved sensitivity.
- We did not mention detailed about the two methods of HCV genotyping because the method section is too long.
- The NAT (COBAS AMPLICORE HCV test, version 2.0, Roche Molecular System) is not a quantitative method and the viral load of HCV could not be considered in this study. We added lack of information of viral load as a limitation in Discussion section. (page 11, line 21-page 12, line 3)

4. [Results] The end of follow was 2008. Some subjects were observed as long as 15 years but some others were followed for only 4 years. Although the person-year used in this study, but the biological effects of each person-year should be different between these two populations. Please draw Kaplan-Meier curves by age of entry and by date (or group) of recruitment.

- We performed Kaplan-Meier curves, but they were not included the manuscript because the main objective of this study is to show the multiplicative synergistic risk on HCC development among co-infected subjects with HBV and HCV

Here are KM graphs for your information.
<By age at enrollment, log scale>
5. [Table 1] In method section, the drinking was graded as 4 categories. In the results, percent of drinking was showed. Which grade and above were counted as drinker?

In the method section, the drinking was graded as 3 categories: no drinking (0 g/day), moderate drinking (0.1-23.9 g/day) and heavy drinking (≥24.0 g/day). Moderate drinking and heavy drinking counted as an alcohol drinker. We re-analyzed drinking by cutoff as heavy drinking (24g/day) and modified the results in Table 2, as per reviewer’s comment number 8. The adjusted RR came to be significant.

6. [Results and Tables] What is “relative risk (RR)” in tables 2~4? If you analyzed your data by Cox model, it should be hazard ratio (HR).
: As per reviewer’s comment, RRs were changed to HRs.

7. [Results and Table 2] You put an unknown group in each variable to prevent cases deleted in the multivariate analysis. The smart method should be mentioned in discussion. However, two points on unknown group should be described and explain. One was variable (FBS in this study) with a high missing rate and the other was variable (ASL in this study) with significant results.
: As per reviewer’s comment, explanation about unknown FBS was added in discussion (page 10, line 24-page 11, line 2). However we failed to explain about significantly elevated HCC risk in unknown AST group.

8. [Results] History of acupuncture and transfusion should be deleted for this HCC study. Based-on your analysis, the drinking might be re-analyzed by cutoff as 24 g/day. It might become significant.
: History of acupuncture and transfusion had shown as a risk factor of HCV infection in the same community (Shin HR et al. Br J Cancer 2000; Shin et al. Hepatol Res 2000), therefore these two variables were included in the analysis although they are not direct risk factors to development of hepatocellular carcinoma. As per reviewer’s comments, alcohol drinking was re-analyzed by cutoff as 24g/day. Please see above number 5.

9. [Results] How did you adjust age in the analyses of tables 2 and 3, as an ordinal or a continuous variable?
: Age at recruitment used in the analyses was ordinal (grouped under 50, 50-59, 60-69 and 70 or older). Please refer to the revised Table 1.

10. [Table 4] HCV genotype 1+2 had much high OR than genotype 1 alone, but their 95% CIs were overlapping. It should be interpreted carefully.
: As per reviewer’s comment, we indicated the overlapping confidence interval in the result section (page 8, line 4-5) and the discussion section (page 11, line 14-15).

11. [Discussion] Please shorten the part on association between chronic hepatitis B & C and HCC.
: As per reviewer’s comment, discussion section was shortened.

12. [Discussion] The key of the study was associated factor of HCC development. Please delete contents which were nothing to do with this study, such as pages 10–11, prevalence change of HBV and HCV, page 11 paragraph 2 HCC screening; page 11 follow-up study.
: As per reviewer’s comment, the descriptions on these issues were deleted in the discussion session.

13. [Discussion] Carefully discuss on HBV vaccine and HCC development. The vaccinee should be too young to be included in your study.
: As per reviewer’s comment, discussion including HBV vaccination was briefly described.

14. [Discussion] Carefully discuss on occult HBV infection. If you checked anti-HBc, the discussion would become results. If not, please use more conservative words.
: As per reviewer’s comment, occult HBV infection is touched briefly.