ANSWERING REVIEWERS

November 28, 2012

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: ESPS-6569-Review.doc).

Title: Association between metabolic factors and chronic hepatitis B virus infection

Author: Chien-Hsieh Chiang and Kuo-Chin Huang

Name of Journal: ***World Journal of Gastroenterology***

ESPS Manuscript NO: 6569

Reviewer Comment #1 (No. 00043561): The present manuscript by Chiang et al. is a summary of available data regarding the relation between HBV infection and metabolic disturbances. However, the content of the work should be enriched by more detailed discussion of the past data by focusing on studies with adjusted results. Because, the main drawback of those studies searching for the variations in metabolic parameters in people infected with HCV or HBV is the lack of adjusted comparisons or ratios. The authors could also discuss the differences and similarities between HBV and HCV infections in this regard. If accepted, the manuscript requires a language check before printing.

Responses:

1. Thanks for your important comment. In our original manuscript, the presentation of odd ratios were indeed adjusted results after controlling for important confounding factors in each cohort cited. We have followed your comment to add more detailed discussion of the past data, as well as some discussions about differences and similarities between HBV and HCV infections. The remarks regarding HCV infections are only concise, as we must be loyal to our assigned topic and put more focus on HBV. Besides, our revised manuscript has been polished by an expert in English language editing.

2. Revisions have been made according to the suggestions of the reviewer as follows:

   Page 4:

   (1) Chronic-HBV-infected university freshers (4475 men and 3751 women) had a higher risk of metabolic syndrome [adjusted odds ratio (OR): 1.58; 95% confidence interval (CI): 1.04–2.47] compared to individuals with seroprotective titers after HBV vaccination[6], after controlling for age, sex, body mass index (BMI), uric acid quartiles, smoking, alcohol consumption, and physical activity.

   (2) However, another population-based cross-sectional study (53 528 participants) showed that the likelihood of developing metabolic syndrome was lower in HBsAg-positive (n = 5995; 12.6%) than HBsAg-negative (adjusted OR: 0.84; 95% CI: 0.76–0.93) adults after controlling for age and sex[7].

   (3) It is worth mentioning that being positive for hepatitis C virus (HCV) antibody was positively associated with reduced high-density lipoprotein (adjusted OR: 1.61; 95% CI: 1.37–1.88), while inversely associated with high triglyceride level (adjusted OR: 0.63; 95% CI: 0.55–0.71) according to the population-based study[7]. Thus, the likelihood of developing metabolic syndrome in HCV carriers (n = 1792; 3.8%) was similar to that in non-HCV carriers.

   Page 5:

(4) There have been controversial results. A hospital-based cross-sectional study (243 men and 264 women; mean age: 46.6 years) showed no significant association of chronic HBV infection with insulin resistance or ultrasonographic hepatic steatosis[8]. Another cross-sectional population study in Hong Kong Chinese reported that HBV-infected individuals (n = 91) had lower intrahepatic triglyceride content measured by proton-magnetic resonance spectroscopy (P < 0.001), lower serum triglycerides (P < 0.001), lower metabolic syndrome (11.0% *vs.* 20.2%; P = 0.034), and lower risk of fatty liver (adjusted OR: 0.42; 95% CI: 0.20–0.88) than controls (n = 922)[9].

Page 7:

(5) Tsan *et al* analyzed a National Health Insurance claims database and found protective effects of statins on HBV- and HCV-related HCC incidence[26,27].

(6) High triglyceride levels (≥ 150 mg/dL) were inversely associated with subsequent HBV-related HCC incidence (adjusted OR: 0.60; 95% CI: 0.40–0.90)[18]. This finding is consistent with the inverse association between high triglyceride levels and high viral load in HBeAg-seronegative patients[15]. HBV X protein was reported to inhibit the secretion of apolipoprotein B[29]; an essential component for the formation of very-low-density lipoprotein (a triglyceride-rich particle). Once HBV actively replicates (high HBV viral load), the HBV X protein increases rapidly and contributes to reduced levels of very-low-density lipoprotein and serum triglyceride levels.

Reviewer Comment #2 (No. 00053727): The present article discusses about the correlation on serum adiponectin levels that were positively related HBV infections in overweight to obese subjects. However the authors should discuss any pathways involved in this. Moreover the authors should discuss about NAFLD/NASH as the components of MS (obesity, hypertension, and dyslipidaemia) are associated with the presence of NASH in patients with CH-B. The presence of hepatic fibrosis seems to be associated with known host and viral factors as well as the presence of abdominal obesity. There needs to be language polishing for better reach to the readers.

Responses:

1. We appreciate your comment. The presence of chronic HBV infection was positively associated with serum adiponectin levels (P <0.0001) (Page 5); while serum adiponectin levels were positively associated with the logarithmic transformation of HBV viral load, but only in HBV-infected subjects being overweight and obese (BMI ≥23 kg/m^2) (P =0.018) adjusted for age, gender, BMI, HBeAg serostatus, liver function, and homeostasis model assessment of insulin resistance (Page 6). We used PPAR γ pathway to explain this phenomenon in our original manuscript. We have added some potential mechanism involved in normal and underweight HBV-infected subjects. The associations of hepatic steatosis, hepatic fibrosis with HBV has been also addressed concisely. Besides, our revised manuscript has been polished by an expert in English language editing.

2. Revisions have been made according to the suggestions of the reviewer as follows:

Page 5:

(1) There have been controversial results. A hospital-based cross-sectional study (243 men and 264 women; mean age: 46.6 years) showed no significant association of chronic HBV infection with insulin resistance or ultrasonographic hepatic steatosis[8]. Another cross-sectional population study in Hong Kong Chinese reported that HBV-infected individuals (n = 91) had lower intrahepatic triglyceride content measured by proton-magnetic resonance spectroscopy (P < 0.001), lower serum triglycerides (P < 0.001), lower metabolic syndrome (11.0% *vs.* 20.2%; P = 0.034), and lower risk of fatty liver (adjusted OR: 0.42; 95% CI: 0.20–0.88) than controls (n = 922)[9].

Page 6:

(2) Liver steatosis was neither associated with HBV viral load in HBeAg-seropositive patients

(adjusted OR: 1.46; 95% CI: 0.90-2.36) nor in HBeAg-seronegative patients (adjusted OR: 0.88; 95% CI: 0.72–1.08).

(3) Consistently, we also revealed that serum adiponectin levels were positively associated with the logarithmic transformation of HBV viral load, but only in HBV-infected patients who were overweight and obese (BMI ≥ 23 kg/m^2) (P = 0.018) adjusted for age, sex, BMI, HBeAg serostatus, liver function, and homeostasis model assessment of insulin resistance[11]. In normal and underweight (BMI < 23 kg/m^2) HBV-infected patients, the activated androgen pathway tended to play a more significant role in influencing HBV viral load than the adiponectin pathway did[14].

Page 7:

(4) Even if some statins might ameliorate steatosis or hepatic fibrosis in humans[28], more debate deserves to be raised because clinicians usually avoid prescribing statins for those with liver enzymes over three times above the baseline, which might confound the real protective effects of statins on incidence of HCC.

Reviewer Comment #3 (No. 00013176): The article is a remarkable source of information about the relationship between metabolic syndrome and adiponectin levels and the evolution of HBV. The article is too concise and needs to be clarified. The results of the various studies cited need to be discussed by the authors and a kind of expert opinion need to be given. The conclusion, which is three-line long leaves the reader lost after the citation of a long list of data.

Responses:

1. Thanks for your important comment. We have followed your suggestions to strengthen our discussion.

2. Revisions have been made according to the suggestions of the reviewer as follows:

Page 8:

The controversy about the association between the presence of HBsAg and metabolic factors should be further elucidated from the perspective of HBV viral load. Obesity was inversely associated with high HBV viral load in HBV carriers with positive HBeAg; while hypertriglyceridemia was inversely associated with high HBV viral load in HBeAg-seronegative individuals. Obesity and hypertriglyceridemia contributed to liver damage independently of inducing HBV replication. The involvement of PPARγ gene expression and might explain why serum adiponectin levels were positively associated with high viral load in overweight and obese HBV-infected patients. Among metabolic factors, diabetes has been the best known to be positively associated with an increased risk of HBV-related HCC. More better-designed long-term prospective research should focus on elucidating association of metabolic factors with chronic HBV infection and its relevant outcomes.

Reviewer Comment #4:

The manuscript should be carefully read as some words are absent. Hence, the sentences do not read well. The structure of adiponectin should be discussed on paragraph 2 on page 4 as the structure of adiponectin confers its bioactivity. The relevance of fibrate to HBV should be discussed on page 6.

Responses:

1. Thanks deeply for your comment. We have moved the sentence about the structure of adiponectin to the assigned location (last paragraph of the section "METABOLIC FACTORS AND HBsAg SEROSTATUS") as suggested (new Page 5). We have also discussed more about the relevance of fibrates to HBV (Page 7). Our revised manuscript has been polished by an expert in English language editing.

2. Revisions have been made according to the suggestions of the reviewer as follows:

Page 5:

(1) The association of HBV with selected adipocytokines is also under investigation. For example, adiponectin is a protein produced by adipose tissue that possesses anti-inflammatory effects, and is inversely related to several metabolic disorders, such as type 2 diabetes[10]. Recently, we demonstrated that, although chronic HBV-infected individuals were heavier than healthy controls, the presence of chronic HBV infection was positively associated with serum adiponectin levels ($P < 0.0001$) and high adiponectin levels over the 75th percentile (adjusted OR: 4.25; 95% CI: 2.36–7.66) after adjusting for age, sex, BMI, and insulin resistance index[11].

Page 7:

(2) High triglyceride levels (≥ 150 mg/dL) were inversely associated with subsequent HBV-related HCC incidence (adjusted OR: 0.60; 95% CI: 0.40–0.90)[18]. This finding is consistent with the inverse association between high triglyceride levels and high viral load in HBeAg-seronegative patients[15]. HBV X protein was reported to inhibit the secretion of apolipoprotein B[29]; an essential component for the formation of very-low-density lipoprotein (a triglyceride-rich particle). Once HBV actively replicates (high HBV viral load), the HBV X protein increases rapidly and contributes to reduced levels of very-low-density lipoprotein and serum triglyceride levels. However, an animal study reported fibrate-induced antiproliferative effects in cultured human HCC cells[30]. The investigators demonstrated that the protective effects were independent of the PPARα pathway. There are still no prospective human studies exploring fibrate use and incidence of HCC in HBV-infected individuals.

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

Sincerely yours,

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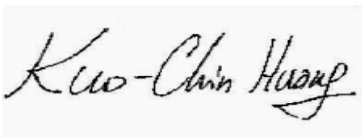

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