Pediatric Gastroenterology and Hepatology, Department of Pediatrics, Chulalongkorn University, Bangkok 10330, Thailand

December 28, 2017

Dear Sir/Madam,

First of all, thank you very much indeed for the reviewer's comments. Enclosed please find a revised version of the manuscript entitle "High prevalence of hepatitis B-antibody loss and a case report of de novo hepatitis B virus infection in a child after living-donor liver transplantation" and the answering reviewers as the following;

Reviewer #1: This manuscript is well written. De novo activation of HBV with escape mutations from hepatitis B surface antibody after living donor liver transplantation had been documented. Circulating antibody to hepatitis B core antigen does not always reflect the latent hepatitis B virus infection in the liver tissue, and to date, latent hepatitis B virus infection in healthy individuals with antibodies to hepatitis B core antigen was discussed. HB core transmission of hepatitis B virus from hepatitis B core antibody-positive donors had been first reported in 1998 [Uemoto S, et al. 1998 Transplantation;65(4):494-9]. If possible, this point will be added.

Response: We appreciate reviewer's comment on these important points of view.

This manuscript is now added **"De novo activation of HBV with escape mutations from hepatitis B surface antibody after living donor liver transplantation had been documented previously"** in the result section, page 13 line 282-284

In the aspect of "Hepatitis B core transmission of hepatitis B virus from hepatitis B core antibody-positive donors" that had been first published by Uemoto S, et al, is now added in the discussion section, page 14-15 line 324-339 with the context of **"Transmission of the hepatitis B virus core from hepatitis B core antibody-positive donors was first reported in 1998 by Uemoto et al[16]. In the present study, HBV existing in the liver of healthy donors who were hepatitis B core antibody-positive, but not in the blood, was shown to be transmitted to recipients by liver grafts following liver transplantation. Moreover, livers from hepatitis B core antibody-positive donors exerted influence on graft survival as this was lower in the recipients of hepatitis B core antibody-positive tissue compared to those receiving tissue from hepatitis B core antibody-negative donors, especially among HBsAg-negative recipients[17]. As a result, robust strategies have been developed to prevent viral activation and *de novo* hepatitis B infection in**

recipients receiving liver grafts from hepatitis B core antibody-positive donors. These strategies involve passive immunization with hyper-immune hepatitis B immunoglobulin (HBIG), with or without antiviral agent, or the administration of hepatitis B vaccine[18-21]. In contrast, the prevention of *de novo* hepatitis B infection in recipients of tissue from hepatitis B core antibody-negative donors has generally been disregarded because evidence to support *de novo* hepatitis B infection from the loss of HBV immunity after liver transplantation is scarce[9, 10].

Reviewer #2: Manuscript ID:37325, entitled, "High prevalence of hepatitis B-antibody loss and a case report of de novo hepatitis B virus infection in a child after living-donor liver transplantation" attempts to indicate the loss of hepatitis B immunity after liver transplantation in children, especially in cases that the liver is from an antiHBc-negative donor. To this end, the article reports an observational study on a 5-year-old boy with biliary cirrhosis who received 4 doses of HBV vaccine with anti-HBs titer of >1000 IU/L and then underwent liver transplantation, wherein donor was his anti-HBc-negative. In addition, the manuscript reviews the potential factors of HBV immunity loss by evaluating the data of 50 children with a mean age of 6.67 and 54% female sex who received primary hepatitis B immunization and had complete data of anti-HBs before and after liver transplantation between May 2001 and June 2017. Finally, it is concluded that loss of hepatitis B immunity, despite high anti-HBs level prior to transplantation is unexpectedly common after liver transplantation. Authors also have concluded that serum anti-HBs, albumin, total bilirubin, and direct bilirubin before liver transplantation were the potential factors of HBV immunity loss after liver transplantation. Accordingly, it is recommended that a booster dose of hepatitis B vaccine and raising serum albumin to the normal level could delay the rapid HBV immunity loss after liver transplantation but might not prevent de novo hepatitis B. Consequently, strategies to maintain anti-HBs antibody above the protective level after liver transplantation are needed. Comments: Taken together, the subject is of interest for audience of WHG journal and the manuscript is well-written and well organized and results are conclusive. The manuscript might further improved by some discussions and comparing with cases when HBcAb positive donor grafts are used (Angelico M et al, Hepatitis B-core antibody positive donors in liver transplantation and their impact on graft survival: evidence from the Liver Match cohort study. J Hepatol. 2013 Apr;58(4):715-23) and strategies to reestablish active immunity against HBV after liver transplantation (Lu SC et al, Reestablishment of active immunity against HBV graft reinfection after liver transplantation for HBV-related end stage liver disease. J Immunol Res. 2014;2014:764234).

Response: We appreciate reviewer's comment to improve our manuscript by discussion more in the view of hepatitis B core antibody-positive liver grafts for the graft survival and the strategies to reestablish active immunity to HBV.

This manuscript is now added these crucial contents in the discussion part as following;

"Transmission of the hepatitis B virus core from hepatitis B core antibody-positive donors was first reported in 1998 by Uemoto et al[16]. In the present study, HBV existing in the liver of healthy donors who were hepatitis B core antibody-positive, but not in the blood, was shown to be transmitted to recipients by liver grafts following liver transplantation. Moreover, livers from hepatitis B core antibody-positive donors exerted influence on graft survival as this was lower in the recipients of hepatitis B core antibody-positive tissue compared to those receiving tissue from hepatitis B core antibody-negative donors, especially among HBsAg-negative recipients[17]. As a result, robust strategies have been developed to prevent viral activation and *de novo* hepatitis B infection in recipients receiving liver grafts from hepatitis B core antibody-positive donors. These strategies involve passive immunization with hyper-immune hepatitis B immunoglobulin (HBIG), with or without antiviral agent, or the administration of hepatitis B vaccine[18-21]. In contrast, the prevention of *de novo* hepatitis B infection in recipients of tissue from hepatitis B core antibody-negative donors has generally been disregarded because evidence to support *de novo* hepatitis B infection from the loss of HBV immunity after liver transplantation is scarce[9, 10].", page 14-15 line 324-339

"However, it is not just strategies to delay HBV immunity loss that are needed; we also need to develop methods to re-establish active immunity against HBV after liver transplantation. Lu et al. reported the improved feasibility of vaccination combined with nucleoside analogues in the prevention of HBV reinfection after orthotopic liver transplantation compared to regular HBIG administration[21]. In this previous study, long-term and repetitive vaccine stimulation was shown to be an important method with which to create and cultivate an enhanced immune response in these immunocompromised patients. Similar to this study, a few pediatric studies have reported the use of intermittent vaccination reinforcement, or booster vaccination, to maintain spontaneous anti-HBs production in children after liver transplantation[10, 25, 26]. For example, Ni Yen-Hsuan et al. studied both the humoral and cellular immunity

of booster hepatitis B vaccines in children after liver transplantation and demonstrated that the immunological response following a booster dose appeared to be adequate, at least over the short term (2 month assessment period)[27]. However, Bauer et al. conducted a pilot study of cellular immune response investigating HBsAg-specific T and B cells in adults after liver transplantation compared with controls and highlighted the role of the strong inhibitory effect of regulatory T cells upon immunological response after hepatitis B revaccination over a period of long term assessment (> 1 year)[27]. The best rationale for HBV revaccination in liver transplantation patients has yet to be elucidated. Future studies are required to identify an appropriate HBV immunization protocol for children after liver transplantation which will effectively re-establish both cellular and humoral immunities to HBV.", page 17 line 391-412

Besides, we highlighted the content we added as reviewers comment with the yellow color and put the red color to the content that editors kindly commented as well. Moreover, English language was critically re-edited and polished again in the whole manuscript by the native English speaker.

Thank you very much indeed again for these useful comments. We hope that our manuscript will be considered to publish in high quality journal as World Journal of Gastroenterology. If there is the point that we can improve our manuscript more, we will feel very grateful for the further comment.

Sincerely yours,
Palittiya Sintusek, MD
Voranush Chongsrisawat, MD