Author's response to reviews

Title: Virologic and Clinical Characteristics of HBV Genotypes/Subgenotypes in 487 Chinese Pediatric Patients with CHB

Authors:

Yanwei Zhong (zhongyanwei@126.com)
Dongping Xu (xudongping@yahoo.com)
Yi Dong (dong1970yi@sina.com)
Xiaoyan Xing (lilyclxy@hotmail.com)
Yu Gan (ganyu1981@163.com)
Dawei Chen (chdw12@163.com)
Fuchuan Wang (wfc_20002000@163.com)
Meilei Gu (gumeilei302@126.com)
Hongmei Tang (hmtang123@163.com)
Shishu Zhu (302zss@sina.com)
Hongfei Zhang (pldc302@126.com)

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Author's response to reviews: see over
Dear Editor of BMC Infectious Disease,

MS. No.1718339911516854 (Virologic and Clinical Characteristics of HBV Genotypes/Subgenotypes in 487 Chinese Pediatric Patients with CHB).

On behalf of all authors, I thank you very much for your favorable response and comments from the reviewers. The comments are instructive. Accordingly, we answer them point by point as follows.

**For comments from Reviewer 1**

1. Precore and core promoter sequences are important to understand virological and clinical features of HBV genotype. Therefore, information of precore and core promoter sequences should be included in this analysis.

   **Answer.** The reviewer’s point is correct. The precore and core promoter sequences have been analyzed in our previous study, and it will be introduced in another article. According to the reviewer’s suggestion, we supplement partial results of precore and core promoter sequences analysis, which we have added in the discussion section in the newly-revised manuscript.

2. Sequenced information in this study cases should be submitted to the DNA Data Bank, and authors state the accession number in the text.

   **Answer.** According to the reviewer’s suggestion, we have supplemented parts of DNA Data with GenBank accession number in the newly-revised manuscript.

3. Some duplication of Reference is seen (number 6 and 37, 34 and 41, 22 and 40).

   **Answer.** We are sorry for the duplication of the References (number 6 and 37, 34 and 41, 22 and 40). The references 37, 40 and 41 have been replaced by correspondingly new references in the newly-revised manuscript.

**For comments from Reviewer 2**

1. Is this study a retrospective cohort study or prospective study? How did the study design? Please describe the inclusion and exclusion criteria. The NA should not be used until the age of the patients are above 16 years in China. Why there are too many pediatric patients used LAM or ADV? Did all of the patients sign the written informed
Answer. This is a prospective study. The study design as follows: Four hundred and eighty-seven pediatric inpatients with CHB (aged 3 to 18 years) who visited Beijing 302 Hospital from September 2007 to September 2010 were enrolled in the study. Including 217 patients who had received 6 months of treatment with interferon-α, but who did not response or not satisfied to IFN-α. The treatment of non-responders continued with LAM. For the patients (aged 12 and older) who did not satisfied to the treatment, ADV was added to the treatment regimen. Informed consent was obtained from each patient’s parent.

According to the reviewer’s suggestion, we have described the inclusion criteria and excluded criteria in the newly-revised manuscript.

According to the reference 11, the approved therapy options for childhood CHB infections are interferon-α (IFN-α) and lamivudine (LAM). According to the reference 4, Lamivudine may be used starting at 3 years of age; Adefovir is labeled for those aged 12 years and older. In this study, 217 patients who had received 6 months of treatment with interferon-α, but who did not response or not satisfied to IFN-α were treated using NA.

2. NA are commonly used in clinic for suppressing viral replication to affect the progression of liver diseases caused by chronic HBV infection. There were two hundred and seventeen patients exposed to nucleos(t)ide analog(s) in this article. The authors should provide the information (treatment drug selection, therapeutic effects, drug-resistance). Theses index would affect the histopathology of the different sub-genotypes patients.

Answer. Thanks for the expert’s question. It is our understanding that this study did not refer to treatment matters, treatment information will be introduced in another article.

3. Among the 487 pediatric inpatients with CHB, 187 HBeAg positive patients who were submitted to liver biopsy. The mean degree of inflammation, stage of fibrosis and ALT level in HBV/C2 patients was significantly higher than HBV/B2 patients (P
< 0.05). But before drawing this conclusion, the factors (such as sex, age, routes of infection, therapeutic effects) affect the degree of inflammation, stage of fibrosis and ALT level of HBV/B2, C2 patients should be excluded. Multiple logistic regression analysis may be used to identify factors related to mean degree of inflammation of patients.

**Answer.** Thanks for the expert’s question. According to the reviewer’s suggestion, we performed multiple logistic regression analysis. The factors (such as sex, age, routes of infection, therapeutic effects) affect the degree of inflammation, stage of fibrosis and ALT level of HBV/B2, C2 patients have been analyzed. The results indicated that there is no statistically significant difference between them. We have described this in newly-revised manuscript. The revised places in the text are underlined and painted red.

**For comments from Reviewer 3**

1. Methods……Line2: about the enrolment the authors have to better specify if consecutive enrolment or if they have design a specific study to avoid sample bias.

**Answer.** Firstly, Setting strict including and excluding criteria of research subjects. The inclusion criteria were: All patients were serum HBsAg positive for at least 6 months, but there was no evidence of HCC, or concomitant of HCV, HDV, and HIV infection, autoimmune liver disease. The exclusion criteria were: Patients with acute hepatitis A, B, HCV, HDV, or HIV co-infection, and drug induced acute hepatitis, existence of renal failure, hepatic decompensation or psychiatric disorders, and central nervous system diseases such as epilepsy, those who had received bone marrow or organ transplants, or had received immunosuppressive, nephrotoxic, or hepatotoxic medications within 2 months of enrollment.


2. In detection of serological marker……Section……Lines 11 and 12: the authors wrote about phylogenetic analysis please delete “evolutionary” because the software used (Mega 4.0) do not give any possibility to make a tree under an evolutionary model but it is for computing the genetic distance only.

**Answer.** The reviewer’s point is correct. According to the reviewer’s suggestion, we have deleted “evolutionary” in the newly-revised manuscript.

3. Analysis of genotypic/subgenotypic drug mutations……Line2 and 3: “substitution……for analysis” have to put in results section they are not methods.

**Answer.** Thanks for the reviewer’s question. As our understanding, the sentence “substitution……for analysis” described the positions rt80, rt173, rt180, rt181, rt204, rt214, rt229, rt233 and rt236, which would be taken as resistance-associated mutations for analysis. So we put it in the methods section.

4. Results……Line 3: please put in bracket the normal level parameter of alanine-aminotransferase.

**Answer.** According to the reviewer’s suggestion, we have put the normal level parameter of alanine-aminotransferase in bracket in the newly-revised manuscript.

5. Results……Line 3 to 5: “the subgenotype distribution……For D”. put in table 1.

**Answer.** According to the reviewer’s suggestion, we have described it according to Table 1 in the newly-revised manuscript.

6. Line 1: “among the……normal level” please put this sentences after the sentence in line 8-9 “The main characteristic……Table1”. The result section have to start with this sentence.

**Answer.** According to the reviewer’s suggestion, we have changed it in the newly-revised manuscript.

7. Please unify table 2 table 3 and table 4 in only one table.

**Answer.** Because the numbers of the samples are different in table 2, table 3 and table 4, we could not put them in only one table.

8. Discussion……Line 6-7: this sentence is very confused, the authors wrote about
genotypes, the have put in methods section the phylogenetic analysis but I do not see any trees, please show the trees built in Mega software specifying the characteristic of the analysis otherwise is not correct to write about genotyping.

**Answer.** According to the reviewer’s suggestion, we supplement a figure (Figure 1) with phylogenetic tree in the newly-revised manuscript.

In methods section, we described the construction of phylogenetic trees for genotyping. Phylogenetic trees were constructed using neighbor-joining (NJ) analysis with bootstrap test confirmation performed on 1000 resampling standard reference sequences were acquired from the online Hepatitis Virus Database which can be found at: http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi. To make the figure differentiable, the 43 representative analyzed HBV genetic sequences with GenBank accession number are presented in Figure 1 in the newly-revised manuscript.

9. In the sentence “the difference……to confirm the conclusion” the comparison between children and adult do not make sense with the paper and the analysis, maybe the authors have to discuss about the not statically results by the point of view of the short time of infection and therapy in pediatric cohort. Moreover I advise to analyze the sequences with phylogeny using the maximum Likelihood criteria because could be interesting to observe if the sequences are intermixed or not by the diseases status to enforce the hypothesis about the implication of C2 genotypes in disease progression also because the author have to take their affirmation with caution because larger population-based study is necessary and more appropriate genetic analysis (i.e. genetic distance between the different genotypes involved in different disease status) are necessary.

**Answer.** The sentence “the difference…… to confirm the conclusion” in the text is not our study result but an inference from the conflicting results of the reference and our study. And additional large population-based studies are needed to confirm this inference. Some inappropriate words have been changed in the new-revised manuscript. According to the reviewer’s suggestion, we have analyzed some relevant data using the maximum Likelihood criteria, but no intermixed sequence was found. This is consistent with the result which we have described in the text.
10. Please check the conclusion and change the strong affirmation with probably or seems that and so on.

**Answer.** According to the reviewer’s suggestion, we have changed some overstated words in the newly-revised manuscript.

The revised places in the text are underlined and painted red, and the language has been corrected by a native-English speaker. The newly-revised manuscript contains 180 words for abstract, 1814 words for text (not including abstract, references and tables), 4 tables, and one figure. We expect that our newly-revised manuscript will make the point. Many thanks for your kind consideration and instructive suggestion from the reviewers.

Thanks again for your time and favorable consideration.

With Best Regards,

Hongfei Zhang, Professor

Director, Pediatric Liver Disease Therapy and Research Center

Beijing 302 Hospital

Beijing 100039, China

Tel/Fax: +86 (10) 63879776; Email: pldc302@126.com