ANSWERING REVIEWERS

Nov. 15, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6132-review.doc).

Title: 'Acute Hepatitis B of Genotype H Resulting in Persistent Infection'

Authors: Norie Yamada, Ryuta Shigefuku, Ryuichi Sugiyama, Minoru Kobayashi, Hiroki Ikeda, Hideaki Takahashi, Chiaki Okuse, Michihiro Suzuki, Fumio Itoh, Hiroshi Yotsuyanagi, Kiyomi Yasuda, Kyoji Moriya, Kazuhiko Koike, Takaji Wakita, Takanobu Kato.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6132

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated to reflect requirements.

2 Revisions have been made according to the suggestions of the reviewer

(1) Reviewer No: 12513

Comment: The manuscript submitted for publication by Yamada et al. is interesting considering a case of chronicity in a patient with HBV genotype infection H. The authors genotyped phylogenetically the circulating HBV by means the direct sequencing of the entire genome, detecting that the genotype is different from that obtained by a routine technique based on an EIA preS2 region epitope. The authors indicate the possibility that routine technologies are not to adequate for HBV genotyping and should be revised in the future. However, in this sense the confirmation with an additional routine technology such as LiPA must be performed.

Response: *As the reviewer suggested, INNO-LiPA HBV Genotyping Kit is the most reliable kit for HBV genotyping and capable of diagnosing the genotype H infection. But, unfortunately, this kit is not common in Japan, and we need much time to obtain it. Therefore, accepting the reviewer's comment, we modified the manuscript as follows;*

1. Deleted the sentence regarding the kits for HBV genotyping.

2. Modified the sentence in discussion as follows; "Presumably, the infrequent use of a reliable and convenient detection kit for genotype H infection has hampered the correct diagnosis of genotype H infection; some cases may be misdiagnosed and considered to be infections by other genotypes. In fact, in the current case, our HBV isolate was originally identified as genotype C by the commercial kit that is covered by insurance in Japan." (Page 10, Line 11).

3. Modified the sentence in discussion as follows; "To this end, the use of a reliable HBV genotyping kit that can correctly distinguish all genotypes is essential for routine clinical practice." (Page 10, Line 18).

Comment: It is indicated the possible association between genotype H (rare in Japan but maybe under-detected by methodological problems as they shown) and the risk of HBV chronicity. In this sense is extremely speculative to draw conclusions from a single case, although it is interesting that raise this possibility.

Response: *We agree that the association of genotype H infection to chronic change is not conclusive. Thus, our argument in this manuscript is that the complete diagnosis of HBV genotyping is important and accumulation of cases of genotype H infection is essential to clarify this association. We modified the last sentence in discussion as follows; "We believe that it is necessary to use kits that are capable of accurate genotyping to permit an accumulation of cases and to investigate the clinical features of genotype H infection in routine clinical practice." (Page 12, Line 2).*

Comment: The work is interesting but has important limitations mainly considering that only the genotype H may be the virologic characteristic associated with chronicity without analyzing with a minimum depth the possible presence of additional virological factors in significant proportions (just direct Sanger sequencing have been performed) in the absence of a clonal study to analyze possible factors as mutations in virologic preS X or Precore / core associated with the process that chronification, or even a evaluation of complexity of viral quasispecies. Without these data the argument about the possible association between genotype H and chronicity are very weak. On this last point it should be added to the discussion a depth analysis of the reported cases of chronifications and its possible association with viral genotypes. In the case there was observed this association it would give great strength to the conclusions of the study. The study clonal sequencing analysis could be accomplished by mass sequencing technology (Ex: 454 Roche or Ion Torrents, it would be much faster and the resolutive than classical cloning. However the latter with a depth of 50 clones would be sufficient since it would allow frequencies in the order of 2-5% of potential minority variants. This analysis must be developed for consider the present study in terms of publication.

Response: *To address the reviewer's suggestion, we amplified the S region by PCR and determined the sequence of 51 clones. Detected sequences were closely related to the consensus sequence with 1 - 3 amino acid polymorphisms. Data was attached as the review only figure, and described in the manuscript as follows; "To assess the complexity of the infecting virus, S region sequences from 51 clones in acute phase serum were determined. The detected sequences were genotype H and were closely related to the consensus sequence determine by direct sequencing with 1 - 3 amino acids polymorphisms (data not shown)." (Page 9, Line 9).*

Minor comments:
Comment: It should be discussed the characteristics of HBsAg detection method used indicating that variants of the surface region is able to detect and compare with other methods such as more widespread Abbott Architect, Johnson Vitros ECI, Siemens Centaur and Elecsys / Cobas Roche. Indicating if the a determinant variant F134L detected in this case is possible to be detected by these other methods.

Response: *We confirmed the disappearance of HBsAg by measuring with ARCHTECT HBsAg (CMIA, Abbott Japan) and obtained consistent data. Thus, we added the sentence as follows; "HBsAg was no longer detected at 4 months from onset by HISCL-2000i. This disappearance was also confirmed by ARCHTECT*

HBsAg (CMIA, Abbott Japan, Tokyo, Japan)." (Page 11, Line 4).

Comment: The introduction is low informative and should include data on both virological and genetic factors which have been proposed as being associated with the process of chronicity.

Response: *To address this reviewer's comment, we added the following sentences in introduction; "There are some differences in the clinical features and outcomes among the genotypes. It has been reported that the persistent infection from acute hepatitis is prevalent in adults that are infected with genotype A HBV. Thus, determining the HBV genotype is of increasing importance even in routine clinical practice, although a reliable kit for determination of all HBV genotypes is still uncommon and is not yet covered by insurance. The host factors associated with persistent infection by HBV have also been reported, such as single nucleotide polymorphisms (SNPs) or genotypes in the HLA-DP locus. It may also be useful for identifying the patients who are prone to develop chronic hepatitis." (Page 6, Line 11).*

(2) Reviewer No: 504486

Comment: The authors reported that HBV genotype H strain from a patient is associated with acute hepatitis following establishment of persistent infection. They also suggested that SNP in the HLA-DP locus is a risk factor of chronic infection. Herein their writing is well organized so that we easily followed what they address. Their finding; HBV genotype H strain is relevant with acute hepatitis as seen other recent reports is worth for the publication of this journal. The finding is accumulating.

Response: *Thank you for your encouraging comment. We hope this report is worthwhile for physicians participating in clinical care of acute hepatitis B outpatients.*

(3) Reviewer No: 6912

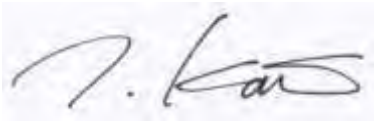
Comment: Dr. Yamada et al described a 47-year-old male with acute hepatitis B which was resulted from hepatitis B virus with genotype H and persisted infection for 26 months at least. Hepatitis B virus genotype H infection is rare in Asia. Therefore, the authors reported this case and claimed that the infection of HBV genotype H could be a risk factor for persistent infection. Acute hepatitis caused by hepatitis B virus with genotype H has been reported in Japan. In this case report, the authors emphasized that hepatitis B virus with genotype H could result in persistent infection. In this case, anti-viral agent was not prescribed for this patient when the patient had acute hepatitis. If anti-viral agents was given, would the clinical course be different?

Response: *In this case, we expected that this case is self-limiting. Because HBsAg was disappeared at 2 months after onset and HBV-DNA titer decreased. However, against our expectation, this patient developed persistent infection. Now we are preparing the administration of anti-viral medication. We added the following sentence in case report; "We are now preparing to administer anti-viral medication." (Page 8, Line 4).*

Finally, the manuscript was edited by American Journal Experts.

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

Sincerely yours,



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Clone23	L.S.	R.	1
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Clone36	S.	G.	1
Clone27	V.		1
Clone29	A.		1
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Clone20	E.		1
Clone52	R.		1
Clone13	S.		1
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Clone46	R.		1
Clone37	P.		1
Clone10	P.		1
Clone11	I.		1
Clone47	E.	E.	1
Clone19		P.	1
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